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Leon J. Cole.

LEON JACOB COLE

1877–1948

LEON J. COLE was born in Alleghany, New York, on June 1, 1877 and died in Madison, Wisconsin, on February 17, 1948. He was a city boy who spent his vacations on the farm during the years of his elementary and high school education. His interest in animals and plants led him first to enter Michigan Agricultural College. In the fall of 1898 he transferred to the University of Michigan where he specialized in zoology and botany. While an undergraduate student, he was chosen to be a member of the Harriman Alaskan Expedition of 1899. His associations with eminent mammalogists, ornithologists and botanists on this expedition intensified and broadened his interest in the natural sciences. He graduated from the University in 1901, served there for a year as a graduate assistant and then went to Harvard University where he received his Ph.D. in 1906 under E. L. MARK. During that period he completed a monograph for the U.S. Fish Commission on "*The German Carp in the United States*," made a zoological expedition to Yucatan and spent a summer at the Bermuda biological station. The other summers from 1901 to 1906 were spent at the Woods Hole Marine Laboratory working for the U.S. Bureau of Fisheries on a biological survey of the region.

In 1906 DR. COLE was appointed Chief of the Division of Animal Breeding and Pathology at the Rhode Island Experiment Station. Although at that time he had no special training in the budding science of genetics, first named by BATESON in the same year, his interest in this field had been stimulated by DR. W. E. CASTLE and other associates at Harvard University. At the Rhode Island Station COLE's first genetic experiments were undertaken with pigeons. This work he continued during a two-year period as instructor in zoology at the Sheffield Scientific School, Yale University, and then throughout the remainder of his academic career.

DR. COLE was called by the University of Wisconsin in 1910 to found, within the College of Agriculture, a new department of instruction and research in plant and animal improvement. This department was first called "Experimental Breeding," but the name was changed to "Department of Genetics" in 1918. Neither the University nor COLE himself wanted any rigid division between fundamental and applied research but rather an intimate combination. It was clear from the beginning that emphasis should be placed on both. Prior to 1910 plant breeding already had established its worth at several European and American institutions, but only sporadic attempts had been made to study inheritance in farm animals from the Mendelian point of view. Nothing was known about the general applications of Mendelism to the improvement of economically important traits in herds and breeds. At Wisconsin the young science of genetics was challenged to show its significance to agriculture in general, in animal as

well as in plant improvement. COLE reacted to this challenge with confidence and dedication, and succeeded in securing the same recognition for advanced training and research in his agro-biological department as already was accorded similar units elsewhere in the University. During the first period COLE had to take care of research and students in plant as well as in animal genetics, but from 1918, he enjoyed the co-operation with a colleague—later several colleagues—specializing in plant genetics, so that he could devote his efforts mainly to the animal field.

In the application of genetics to animal breeding, LEON J. COLE was a pioneer, and for many years the leading authority. Probably none would have been better qualified, especially in the early years, for the difficult task of combining an unwavering scientific approach to the problems with a keen interest in, and a deep understanding of, the practical implications. His broad background in general biology made him realize at an early stage that animal genetics was far more complicated than the simple Mendelian ratios obtained in studies of qualitative characters such as horns or color seemed to suggest. Because of his unpretentious and pleasing personality, it was easy for him to make contacts and to establish co-operation with practical breeders and their organizations as well as with colleagues working in allied fields of science, a co-operation which is often needed in animal breeding investigations.

DR. COLE believed in giving his students a considerable latitude of choice for a doctorate problem. Therefore, he maintained a variety of materials for their use, e.g. various laboratory animals, rabbits, pigeons, poultry and cattle. From his early years as a naturalist he retained a great interest in ornithology, and research on the genetics of pigeons and doves became a continuous thread through his work at Wisconsin. Sex linkage was encountered in the pigeons in 1912, and Mendelian analyses were made of the inheritance of the principal colors. Interspecific transfer of a gene for feather pigmentation from *Streptopelia chinensis* to the common ringdove was effected, giving a dark form of the latter previously unknown in domestication. COLE was also intensely interested in the problems of evolution and the fertility in hybrids. Cytological investigations showed that the abnormal sex-ratio from crosses between pigeons and ringdoves was due to differential mortality of the female embryos. For further exploration of species differences, he encouraged DR. M. R. IRWIN, added to the staff in 1930, to take up immunogenetic studies of the species relationships in Columbidae. Under DR. IRWIN's leadership, blood group studies were undertaken also on cattle, sheep, pigs and poultry, making the Department of Genetics at Wisconsin a world center for investigations on the immunogenetics of farm animals.

Significant early contributions were made by COLE and his graduate students to the genetics of poultry. Together with colleagues in the Poultry Husbandry Department, he made the first systematic experiments on inbreeding in chickens. Considerable work was done also to test the alleged possibilities of controlling sex in mammals, all with negative results. In 1912, a crossbreeding experiment with cattle was started to study the inheritance of dairy and beef qualities. The Aberdeen-Angus were crossed to Jerseys and later to Holstein-Friesians, an F_2

generation was raised and some backcrosses were made. Although, for economic reasons, this experiment could never be carried out on a scale large enough to provide decisive results about the inheritance of milking ability or composition of the milk, it yielded valuable information in several respects. COLE also studied the inheritance of lethal and other defects in farm animals as well as the genetic basis of disease resistance. After he retired from the chairmanship of the Department in 1939, he devoted much of his time to investigating the genetics of color phases in the fur animals, especially foxes and mink; together with one of his graduate students, R. M. SHACKELFORD, he published several papers on this subject.

Due to his broad interests and wide personal contacts, LEON J. COLE became engaged in much work outside the Department of Genetics, although in the realm of animal genetics. In 1923, he took a leave of absence from Wisconsin, and for a year was Chief of the Animal Husbandry Division of the Bureau of Animal Industry of the U.S. Department of Agriculture. In that capacity, he reinforced materially the activities of the Division and stimulated work on more fundamental problems at the various branch stations of the Division. In 1925, DR. COLE was elected Vice-Chairman of the Division of Biology and Agriculture of the National Research Council and was Chairman of the Division for 1926–1927. For several years, he was Chairman of the Committee on Animal Breeding which had been formed by the National Research Council for stimulating and co-ordinating breeding research. DR. COLE also represented agriculture in the Board of Biological Fellowships of the National Research Council. In this capacity, he assisted many promising young men to receive further scientific training, a cause to which he was deeply devoted. He was always ready to give of his time generously to scientific organizations and to the promotion of science at large. He was President of the Wisconsin Academy of Arts and Sciences from 1924 to 1927, and was President of the Genetics Society of America in 1940. From 1935 until his death, he was a member of the Editorial Board of Genetics. He served for many years on the Advisory Committee and later on the Council of the American Genetic Association, and on the Council of the American Naturalist. He received several recognitions for his achievements in teaching and research from his own country and also from abroad; only two will be mentioned here. In 1945, he was awarded the honorary Doctorate of Science by the Michigan State College of Agriculture and Applied Science. In 1939, the American Society of Animal Production presented his portrait to hang in the noted gallery of the Saddle and Sirloin Club at Chicago.

The impact which LEON J. COLE has made on the development of a scientific basis of animal breeding, through his own work and, not the least, through his training of graduate students from all parts of the world, can hardly be overestimated. While he was Chairman of the Department of Genetics (1910–1939), 61 masters degrees and 62 Ph.D. degrees were conferred upon students majoring in Genetics, and more than half of those were given in animal genetics. He continued to supervise the training of graduate students in his own field until his final illness made that impossible. Most of the leading American workers in

the field were his students until the late 1930's, when they, in their turn, guided the training of the next generation of researchers. DR. COLE's graduate students all carry with them the memory of a scientist and teacher with a clear and penetrating mind, always seeking the truth honestly and straightforwardly without preconceptions and without any poses of personal authority, always exceedingly generous and willing to give the help and advice that was needed. The Department of Genetics was a family of professors and students where lasting friendships were established, and DR. COLE's hospitable home was the center of that family.

IVAR JOHANSSON

GENETIC EFFECTS OF CHRONIC X-IRRADIATION EXPOSURE IN MICE[1]

DONALD R. CHARLES, JOSEPH A. TIHEN, EILEEN M. OTIS, AND ARNOLD B. GROBMAN

Received September 19, 1960

IN 1943 the available data concerning the type and extent of genetic change induced by X-rays or similar high energy irradiation had been derived mostly from experiments on small organisms remote from man, and from the use of single, brief and high exposures. Data relevant to the probable effect on humans of repeated or chronic low level exposure were virtually nonexistent; persons concerned with the setting, maintenance and possible revision of exposure limits were in urgent need of such data.

It appeared that experiments should be conducted that would involve the use of mammals and that would also involve levels and rates of exposure approximating those that might be received by persons working near X- or gamma-radiation sources. Numerous considerations dictated the choice of *Mus musculus* as the experimental animal. The need for such an experiment was recognized by DR. STAFFORD L. WARREN in planning the medical investigations of the Manhattan Project, and the responsibility for its design and execution was entrusted to the late DR. DONALD R. CHARLES. The work was conducted during a period of nearly seven years, from late in 1943 until about 1950, at the University of Rochester School of Medicine under the auspices of the wartime Manhattan Project and, later, its successor, the Atomic Energy Commission. In its execution over 9,000 man-days have been spent and over 400,000 animals bred.

The initial phase of the experiment was completed by the summer of 1946; all of the offspring of control and irradiated male mice had been produced, raised, bred (female offspring only) and autopsied. Preliminary tentative analyses of some of the results were already under way at that time. Genetic testing of the progeny of selected offspring of irradiated males continued until late 1950. Numerous test pedigrees were produced and examined, and an attempt at analysis of their genetic nature was begun during this period.

Following completion of the actual experimental work, the late DR. CHARLES continued the task of collating and analyzing the accumulated data for the purpose of preparing a final report. His death intervened in November, 1955, before this task could be completed. At the instigation of DR. GROBMAN and DR. HENRY

[1] This paper is based on work performed under contract with the United States Atomic Energy Commission.

The paper is a resume of the complete work of the same title issued as University of Rochester Atomic Energy Project Report, UR-565. For information on the availability of this report, inquiry should be made to "University of Rochester Atomic Energy Project, Box 287, Station 3, Rochester 20, N.Y., Attention: Library."

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A. BLAIR, several of the professional personnel who had originally participated in the experiment were again assembled at the University of Florida in 1957 to re-examine the data still available. From these data, and from outlines and a partial typescript previously prepared by DR. CHARLES, a final report was completed. This report was submitted to the Atomic Energy Commission on September 14, 1958, and is currently being processed for reproduction and distribution at the University of Rochester as "University of Rochester Atomic Energy Project Report UR-565."

METHODS

The experimental procedure may be briefly outlined as follows: Male *dba* mice were subjected to chronic low-dosage X-irradiation at rates of 0.1r/day (58 individuals), 0.5r/day (29 individuals), 1.0r/day (16 individuals) and 10.0r/day (33 individuals). There were also 51 control males, not exposed to radiation, but handled in exactly the same manner as the experimental males in all other respects. These 187 males sired a total of over 12 000 offspring, representing a like number of sperm. The offspring were examined and counted at birth and at weaning and were then raised to maturity. Females were bred to determine fertility, and both males and females were subjected finally to a thorough autopsy, in an attempt to determine to what extent, if any, the sperm had been damaged so as to produce a detectable effect in these immediate offspring of the treated males. Many individuals exhibiting abnormalities of various sorts were progeny-tested to determine whether or not there was a genetic basis for the abnormalities. In many other instances, however, (e.g., abnormalities discovered in autopsy of males) such tests could not be conducted. Recessive mutations, which would become apparent only in subsequent generations, would not be systematically detected by the standardized procedures employed.

RESULTS AND DISCUSSION

Although the experiment concerned primarily the genetic effects of radiation, a few observations were made of direct effects on the exposed parent males. Survival time was generally slightly lower in exposed than in control animals, the difference being demonstrably significant, however, only at the 10.0r/day level. Fecundity of the parent male was, as would be expected, greatly reduced at the 10.0r/day level also, 50 percent becoming sterile after about 30 treatments; no litters were produced by any male exposed at this level after more than 100 treatments had been received.

The autopsies produced very extensive data on morphological variability in presumably isogenic individuals. Very few of these data bear directly on the question of radiation effects; we feel, however, that they are of considerable value in their own right and as possible background information for study of many genetically influenced morphological characteristics. Such data are, therefore, included in the report in considerable detail, though they will not be discussed here.

Apparent genetic damage to the immediate offspring of the irradiated males was recognized in three different ways. One was an increased juvenile mortality, that is, a greater mortality rate between birth and weaning. The differential in mortality appears to be influenced by the same sort of environmental factors (e.g., litter size) that influence the juvenile mortality rate in general. The amount of excess mortality, then, is not a function of paternal irradiation alone, but varies also with other factors completely independent of the amount of radiation. A single over-all figure relating increased juvenile mortality to paternal irradiation is, consequently, subject to considerable qualification. Nevertheless, the average magnitude of the effect, under the average conditions obtaining in this experiment, could be computed as an increase in mortality rate of .05 per thousand individuals born (5.0×10^{-5}) per r-unit of paternal irradiation.

Abnormalities for which genetic tests were conducted included both visible morphological features and aberrations of fertility and/or sex ratio. In some instances the tests were inconclusive, either because of possible random loss of a mutation when few progeny were available, possibilities of contamination in certain instances, and other factors that need not be discussed fully here. However, such inconclusive tests were distributed proportionally among the offspring of control and experimental animals. Whether these are treated as positive or as negative test results, one arrives at essentially the same figure for genetically tested and proven induced mutations, including, under this term, chromosomal aberrations as well as visible mutations. The calculated induced mutation rate (dominant mutations only are considered) was 4.5×10^{-5} per r-unit of paternal irradiation.

Among the abnormalities observed but not, in most instances, subjected to genetic testing, there were, in general, no specific abnormalities that appeared more frequently among the offspring of treated than of control animals. However, when all "rare" (defined as occurring in no more than four of the approximately 12,000 individuals autopsied) abnormalities were grouped together, it was found that a significantly greater proportion of such abnormalities appeared among the offspring of exposed males. When related to amount of paternal exposure, the average increase in number of animals with rare abnormalities was computed at 2.4×10^{-5} per r-unit of paternal exposure.

Combining the figures derived from these three sources, with appropriate allowance for a certain amount of duplication, we arrive at a total induced rate of damage of 1.16×10^{-4} per r-unit of paternal exposure. This figure is subject to many qualifications that cannot be discussed at length here, but that are treated in the original report. With the numbers involved, the possible sampling errors are of considerable magnitude, and all figures are at best only rough estimates. Any departure from linearity with respect to either intensity of dosage or number of treatments could not be determined from the numbers available, even if it existed. There is, however, no indication of a threshold effect of any sort; in fact, the apparent effect in mice treated at 0.1r/day was greater than would be expected on the basis of total dosage alone; this could not be shown to be statistically significant and may have been due to sampling error. It is to be noted further

that, whatever the accuracy of the rates of damage determined, they apply only to damage that was detected in the immediate offspring, i.e., dominant mutations; many minor morphological abnormalities, or physiological changes of varying degrees of importance, as well as any possible decrease in life expectancy other than that expressed as juvenile mortality, would undoubtedly have been overlooked. Further, no attempt whatsoever was made to detect the presumably more numerous and, from a long range population viewpoint potentially more serious, recessive mutations that would be expressed in future generations.

SUMMARY

A full description of an experiment involving chronic exposure of mice to X-irradiation at dosages varying from 0.1r to 10.0r daily is contained in University of Rochester Atomic Energy Project Report UR-565. The present paper briefly describes the contents of this report. Genetic damage to the immediate offspring of the irradiated mice was detected in the forms of increased juvenile mortality, increased occurrence of rare morphological abnormalities, and progeny-tested mutations affecting both morphology and fecundity. Several factors, notably sample sizes, render accurate quantitative interpretation of the experimental results difficult, but some damage appeared demonstrable at even the lowest levels of treatment.

EXPERIMENTS ON HYBRID SUPERIORITY IN DROSOPHILA MELANOGASTER.

I. EGG LAYING CAPACITY AND LARVAL SURVIVAL

GERT BONNIER

Institute of Genetics, University of Stockholm, Stockholm, Sweden

Received March 9, 1960

IN the present paper experiments are described on the capacity of egg production and of the sensitivity to temperature stresses in *Drosophila melanogaster*. A temperature of 25°C may usually be taken as an optimal temperature for this fly; with an increase of a few degrees, larvae and pupae meet markedly increased difficulties to survive. In order to get enough material, oviposition and hatching of the eggs were allowed to take place at 25°C in all cases, The sensitivity to temperature stresses is measured by the proportion of larvae which survive to eclosion, and this is made for two temperature levels *viz.* 25°C and 30°C.

MATERIAL AND METHODS

All flies emanated from three wild type stocks. One of the stocks was American (Oregon), and two were Swedish (Karsnäs and Skaftö); they will be symbolized as stocks E, K, T, respectively. From each of these a number of strains, homozygous for the three major chromosomes, were produced, and one strain from each of the stocks was kept for the experiments; they will be symbolized as strains e, k, t, respectively. The method used in the process of homozygotization is shown in Figure 1. As may be seen, the homozygous strains got their chromosomes 1, 2, 3, and Y from the corresponding stock. The generation on the bottom line in Figure 1, i.e., the first generation in which homozygous wild type flies were mated, will be taken as generation 0. The generations following it will, consequently, be regarded as generations 1, 2, 3...

The homozygous flies of strains e, k, t were, in generation 0, few in number, and it was necessary to propagate them for some generations. It is known (MULLER 1954) that spontaneous mutations occur in early embryonic life, during the period of meiosis, and in ageing spermatozoa; during ageing of the flies themselves, however, practically no mutations occur. Because the experiments should be made on a rather large scale all crosses and tests could not be made simultaneously. They were, therefore, repeated in ten runs starting on ten consecutive weeks. In order to minimize the occurrence of spontaneous mutations the following procedure was in principle adopted. Some of the flies from generation 1 were propagated for four generations. In this, and all other propagations, females were allowed to oviposit during a maximum time of four days after their first matings. Other flies of generation 1 were aged for two weeks. In this, and all subsequent

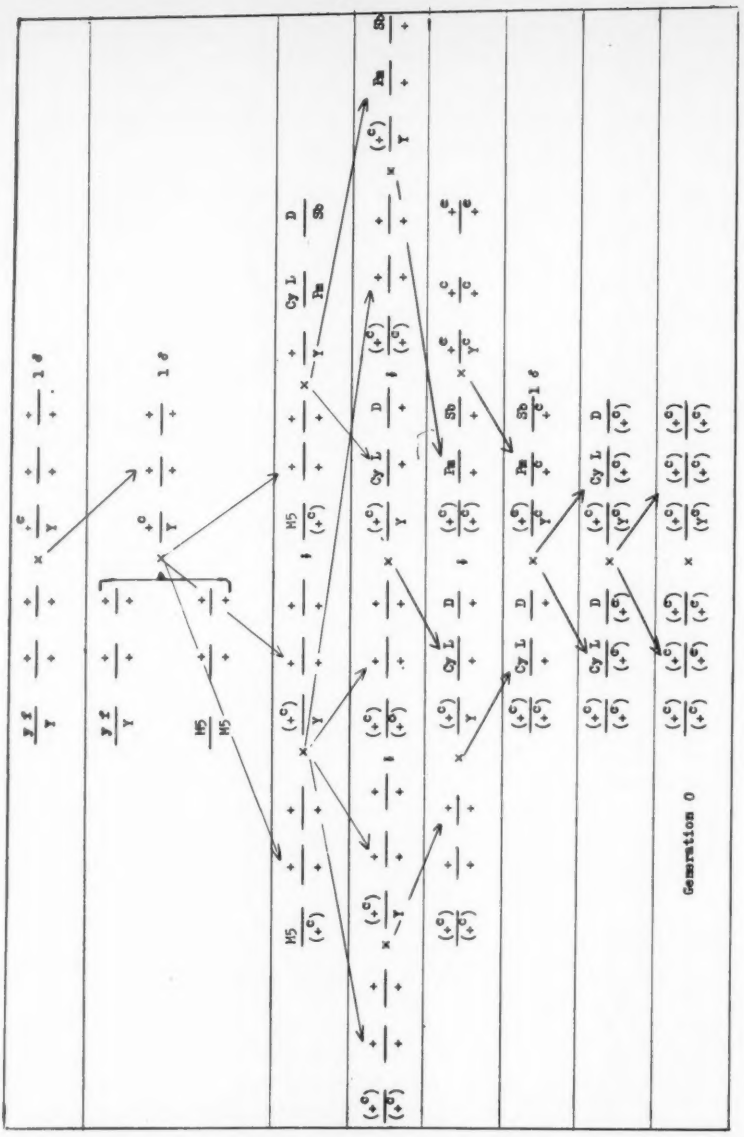

FIGURE 1.—Method used for producing homozygous strains which are isogenous for chromosomes 1, 2, 3, and for Y. The female parent is in all crosses to the left and the male parent to the right. Those wild type chromosomes made homozygous are shown within parenthesis. The letter c indicates that the chromosome emanates from the specific wild type stock used. Females with X chromosome *y f*/Y are so-called double attached with an extra Y chromosome. M-5, *Cy L, Pm, D*, and *Sb* are crosssing over reducing dominant markers. The method avoids the use of females which are heterozygous, simultaneously, for M-5 and for the second and third chromosome markers. The use, in the second chromosome, of both *Cy* and *L* gives a good guarantee that crossovers would be detected. *D* covers fairly well the whole of chromosome three.

ageings, the females were aged as virgins, and the males were kept with other females during their ageing. After ageing, females and males were mated. One part of the progeny was propagated for three generations and one part aged for two weeks. Following this system, a large number of wild type flies was produced on five occasions between each of which two weeks had elapsed; all of these flies belonged to generation 5. Each of the lot of flies so produced was used for starting two consecutive runs of the experiment. The ten runs could, hence, more correctly be said to be made up of five double runs.

The stock flies E, K, T were for each double run taken afresh from the laboratories' stock bottles. In order to get enough material to start the runs using E, K, T simultaneously with the runs using e, k, t, the E, K, T were propagated for some generations in advance.

Experiments were performed with flies e, k, t and their six possible hybrids (including reciprocal crosses); and also with flies E, K, T and their six possible hybrids. No crosses were made between E, K, T and e, k, t. Table 1 shows the different items of one run, exemplified for flies e and for crosses between e females and k males. The runs with the other strains and crosses were performed in an identical manner.

TABLE 1

Description of one run of the experiment, using as example e flies and e females mated to k males. All other genotypes were treated in exactly the same way. The left part of the table contains matings made for a study of the egg laying capacity of pure females and of the survival of pure and hybrid larvae. The right part contains matings made for a study of the egg laying capacity of hybrid females

Item	Length of time between consecutive items	Content of items	
1		Beginning of collection of e females and e males of generation 5 and keeping them together in vials.	Beginning of collection of virgin e females and k males of generation 5 and keeping females and males in separate vials.
	7 days		
2		Transferring the flies to cages with food in Petri dishes.	Mating e females to k males in cages with food in Petri dishes.
	3 days		
3		Inserting Petri dishes with black food into the cages.	Inserting Petri dishes with black food into the cages.
	16 hours		
4		Removing the Petri dishes from the cages.	Removing the Petri dishes from the cages.
	28 hours		
5		Collection from the Petri dishes of freshly hatched larvae of generation 6 and transferring them to vials: 75 larvae per vial.	Collection from the Petri dishes of freshly hatched larvae of generation 6 and transferring them to vials: 75 larvae per vial.
	8 days		
6		Collecting e females and e males and keeping them together in new	Collecting hybrid females and males from the cross e female by

TABLE 1—Continued

Item	Length of time between consecutive items	Content of items	
		vials. Collecting virgin e females and k males and keeping females and males in separate vials.	k male and keeping them together in new vials.
	2 days		
7		Transferring the non virgin e females and e males to new vials, each vial with one female and two males. Transferring the virgin e females and k males to new vials, each vial with one female and two males. 30 vials were made up and numbered from 1 to 30.	Transferring the flies to new vials, each vial with one female and two males. 15 vials were made up and numbered from 1 to 15.
	4 days		
8		The flies of the 25 first vials in which the female still was alive were transferred to 25 empty vials into which a spoon with black food was inserted.	The flies of the 13 first vials in which the female still was alive were transferred to 13 empty vials into which a spoon with black food was inserted.
	16 hours		
9		Flies removed from the vials. The number of eggs counted on each spoon separately. This was made on the spoons of the 20 first vials in which the female still was alive.	Flies removed from the vials. The number of eggs counted on each spoon separately. This was made on the spoons of the ten first vials in which the female still was alive. (End of the experiments on the egg laying capacity of hybrid females.)
	28 hours		
10		Freshly hatched larvae of generation 7 collected from the spoons and transferred to vials, 25 larvae per vial. This was not made separately from each spoon but collectively from the 20 spoons containing larvae of the same genotype. Half the number of vials so produced was incubated in 30°C and the other half in 25°C.	
	7 days		
11		*30° series* First count of adults. All vials containing the same genotype were counted collectively.	*25° series*
	1 day		
12			First count of adults. All vials containing the same genotype were counted collectively.
	3 days		
13		Second (= final) count of adults.	
	1 day		
14			Second (= final) count of adults.

As may be seen, items 1 to 4 include flies belonging to generation 5, and items 5 to 9 include larvae and flies of generation 6. The capacity of egg production in the case of homozygous flies was, therefore, tested on females of generation 6. The reason for not testing this capacity one generation earlier was to secure, as far as possible, similar conditions for all compared genotypes, including conditions during larval growth. Table 1 shows also that it was the larvae of generation 7 which were tested with regard to their capacity of reaching the adult stage at 25°C and at 30°C.

The amount of food in the vials (Table 1) was always about 12 ml per vial (though not measured in individual cases).

RESULTS

Treatment of the results consists in most cases of a comparison of nonhybrids with hybrids. Flies from the E and K stocks (i.e. "pure" flies) are, for instance, each compared with their two reciprocal hybrids. This gives four comparisons. In the same way one gets four comparisons from E and T, and, likewise, four from K and T. Hence there are 12 comparisons to be made with the stock flies. But as the studies comprise only nine groups of stock flies, all 12 comparisons are not independent. This must, of course, be borne in mind when judging the results of the statistical analyses.

Egg laying capacity: Item 6 of Table 1, in the left column, shows that the e females which were intended for crossing with k males were, for several days, kept as virgins. This was not the case with the hybrids shown in the right column, which from their eclosion were kept together with males. Therefore, these hybrids may, in a more satisfactory way, be compared with the pure e flies which, according to the left column, also were kept with males from the beginning of their adult life. Comparisons of egg production capacities are, hence, confined to these kinds of groups. Item 9 of Table 1 indicates that 20 of the pure females and ten of the hybrids were tested for each run. However, in spite of the margins used (see items 7 and 8) there were some instances in which less than 20 females were alive to be tested. Moreover, flies of the E stock, in which the females were very poor in egg laying capacity, were not available for the first double run (runs 1 and 2).

The results of the egg counts are shown in Table 2 in which also coefficients of variation are given. Stock E females are, by far, the poorest layers. Hybrids produce more eggs than pure females (Table 2). In the case of homozygotes there is only one comparison in which the homozygote did produce more eggs than one of the hybrids (Table 4), though quite insignificantly so (k *versus* hybrids from e females crossed to k males). Of the remaining 11 comparisons with homozygotes, three are without statistical significance, whereas eight are significant. All hybrids between stock flies produce more than the pure ones, which is in good agreement with observations made by GOWEN (1952). Among the 12 comparisons there is only one in which the probability level exceeds the five percent point (T *versus* hybrids from E females crossed to T males). None of the three com-

TABLE 2

Average number of eggs produced by single females during 16-hour periods of oviposition

Female parent	Male parent	Number of females tested	Number of eggs	Coefficient of variation	
				Within runs	Between runs
e	e	191	18.5 ± 3.9	0.92	0.90
e	k	100	46.4 ± 6.6	0.45	0.51
e	t	100	58.4 ± 5.8	0.32	0.30
k	k	200	47.0 ± 4.8	0.46	0.27
k	e	100	64.6 ± 5.4	0.26	0.17
k	t	100	67.5 ± 5.6	0.26	0.16
t	t	197	46.9 ± 4.7	0.44	0.31
t	e	100	64.3 ± 5.9	0.29	0.35
t	k	100	57.6 ± 6.1	0.34	0.38
E	E	160	9.9 ± 3.9	1.77	0.52
E	K	80	56.5 ± 7.0	0.39	0.37
E	T	80	64.8 ± 6.1	0.30	0.14
K	K	200	37.7 ± 5.1	0.60	0.40
K	E	100	60.4 ± 7.6	0.40	0.32
K	T	100	69.7 ± 6.9	0.31	0.25
T	T	184	48.3 ± 6.7	0.53	0.34
T	E	100	66.8 ± 5.8	0.28	0.28
T	K	100	70.2 ± 6.2	0.19	0.22

Using the symbols s^2_w = mean square within runs; s^2_b = mean square between runs; n = number of females tested per run; $\bar{x}$ = general average, the following formulae are used:

$$\text{standard error of mean} = \sqrt{\frac{s^2_w}{n}}$$

$$\text{coefficient of variations within runs} = \frac{\sqrt{s^2_w}}{\bar{x}}$$

$$\text{coefficient of variations between runs} = \frac{\sqrt{\frac{s^2_b}{n}}}{\bar{x}}$$

In the few cases where there was unequal numbers of females per run, n is substituted by the harmonic mean of the different n:s.

parisons between stock flies and their corresponding homozygotes give statistically significant differences.

The coefficients of variation within runs (Table 2) are larger for pure females than for hybrids. This may to some extent also be true for coefficients of variation between runs, though not as conspicuously as for those within runs.

Larval survival in 25°C and 30°C: Experiments testing the possibility of the larvae to survive to the adult stage at 25°C and 30°C are described in items 10–14 of Table 1. The number of eggs varied very much between the different genotypes as well as between runs, and as the larvae were collected in groups of 25 (Table 1, item 10) they varied from run to run. The results arrived at after the last run are given in more detail in Table 3.

The primary purpose of the present investigation was to study the temperature sensitivity, and, as a measure of this sensitivity, the proportion was chosen of the number of freshly hatched larvae which, under the given circumstances, were able to reach adult stage (Table 1, items 10–14). From Table 1 (items 11–14)

TABLE 3

Larval survival in 25°C and in 30°C. Total material

Female parent	Male parent	Number of larvae collected for rearing in 25°C	Number of larvae collected for rearing in 30°C	Proportion of larvae reaching adult stage in 25°C (p_{25})	Proportion of larvae reaching adult stage in 30°C (p_{30})	p_{30}/p_{25}
e	e	1225	1225	0.878	0.816	0.929
e	k	1400	1400	0.952	0.908	0.954
e	t	1400	1400	0.966	0.929	0.962
k	k	3750	3750	0.970	0.746	0.769
k	e	1475	1500	0.942	0.950	1.008
k	t	2675	2675	0.945	0.938	0.993
t	t	3225	3225	0.945	0.872	0.923
t	e	1850	1875	0.956	0.922	0.964
t	k	2675	2675	0.977	0.939	0.961
E	E	525	500	0.930	0.784	0.843
E	K	775	800	0.956	0.945	0.988
E	T	425	425	0.962	0.936	0.973
K	K	3075	3075	0.943	0.878	0.931
K	E	950	975	0.947	0.962	1.016
K	T	2675	2675	0.973	0.935	0.961
T	T	3325	3350	0.933	0.913	0.979
T	E	1200	1225	0.962	0.935	0.972
T	K	3175	3175	0.960	0.959	0.999

it is seen that all adults of each separate genotype were counted collectively. This was done because otherwise the routine work would have taken too much time. But, as a consequence, variation within genotypes could not be observed. Hence, the only part of the variance which in the statistical analysis could be estimated was that due to random (binomial) causes. It should be observed, however, that the experiments for each run were performed simultaneously for all genotypes (though with the exception that there were no E flies available at runs 1 and 2). This will tend to cancel environmental differences between the genotypes. Hence, if, in a comparison, there is no real difference between two genotypes the variance of the difference between the genotypes would be equal to the sum of the random variances. An ordinary t test, based on the difference between the genotypes and on the sum of their randomly caused variances, would, as a rule, give a reliable statistical test. It is now known that a proportion, p, is distributed sufficiently close to normality if np (n being the number on which p is based) exceeds a certain value, e. g. 15, and that the same holds good for $n\ (1 - p)$. Let now n_{25} and n_{30} be the total number of larvae collected from a certain genotype for rearing in 25°C and 30°C, respectively, and let f_{25} and f_{30} be the number of larvae reaching the adult stage, and denote the proportions of these larvae, f_{25}/n_{25} and f_{30}/n_{30}, by p_{25} and p_{30}. As these proportions in most cases are close to the limit 1, it is necessary for the analyses that $n_{25}\ (1 - p_{25})$ and $n_{30}\ (1 - p_{30})$ each exceeds, say, 15. But $n_{25}\ (1 - p_{25}) = n_{25} - f_{25}$ and $n_{30}\ (1 - p_{30}) = n_{30} - f_{30}$. We must, therefore, have $n_{25} - f_{25}$ and $n_{30} - f_{30}$ larger than 15. This is the case for all

TABLE 4

Summary of significance tests

Type of test	Homozygotes versus their hybrids: Number of cases in which homozygotes < hybrids	homozygotes > hybrids	significance	Stock flies versus their hybrids: Number of cases in which stock flies < hybrids	stock flies > hybrids	significance	Homozygotes versus stock flies: Number of cases in which homozygotes < stock flies	homozygotes > stock flies	significance
Egg production	8	..	+[1]	11	..	+[5]	..	2	—
	3	.	—	1	..	—	1	..	—
	..	1	—	..	..	..	..	..	..
Larval survival in 25°C (p_{25})	6	.	+[2]	9	..	+[6]	1	..	+[9]
	2	.	—	3	..	—	..	1	+[10]
	..	3	+[3]	..	..	..	..	1	—
	.	1	—	..	..	..	..	..	..
p_{30}/p_{25}	10	..	+[4]	9	..	+[7]	2	..	+[11]
	2	..	—	..	2	—	..	1	+[12]
	..	..	..	..	1	+[8]	..	..	..
	24	..	+	29	..	+	3	..	+
Total	7	.	—	4	..	—	1	..	—
	..	3	+	..	1	+	..	2	+
	..	2	—	..	2	—	..	3	—

	P between 0.05 and 0.01	*P* between 0.01 and 0.001	*P* less than 0.001	*P* between 0.05 and 0.01	*P* between 0.01 and 0.001	*P* less than 0.001	*P* between 0.05 and 0.01	*P* between 0.01 and 0.001	*P* less than 0.001
1)	3	1	4	5) 5	.	6	9) .	.	1
2)	.	.	6	6) 3	2	4	10) .	.	1
3)		1	2	7) 1	1	7	11) .	.	2
4)	1	1	8	8) 1	.	.	12) .	1	.

When the significance level, *P*, is equal to or less than 0.05 this is indicated by the sign +; otherwise by the sign —. The distribution of the observed magnitudes of *P* is, in cases of significance, shown at the very bottom of the table.

genotypes when using the whole material (Table 3), i.e., the material available after ending the tenth run. The analyses are, hence, based on the total material.

When analysing proportions, the computations are often much simplified by substituting, for the proportions themselves, their natural logarithms. Moreover, by using logarithms, a possible metric bias due to proportionality between variances and averages would probably be minimized. It is now found that

$$\text{variance of log nat } p_{25} = \frac{n_{25} - f_{25}}{n_{25} \cdot f_{25}}$$

and

$$\text{variance of log nat } p_{30}/p_{25} = \frac{n_{30} - f_{30}}{n_{30} \cdot f_{30}} + \frac{n_{25} - f_{25}}{n_{25} \cdot f_{25}}$$

Table 4 contains the significances which are based on t tests for differences of log nat p_{25} and for differences of log nat p_{30}/p_{25}.

Looking now at p_{25}, the larval survival in 25°C, for the homozygotes (Table 3) it is found that larvae from e have a significantly poorer survival than three of its hybrids (in two of which the differences are significant); while larvae from k have a significantly better survival than three of its hybrids. Turning to the stock flies and their hybrids it is seen that larvae from the pure stocks in all cases are inferior to their corresponding hybrids; in nine of the 12 comparisons the differences are significant. Comparing, finally, stock flies with homozygotes, one finds that larvae from E are significantly superior to those from e; that larvae from K are significantly inferior to those from k; and that larvae from T are inferior to those from t, but only on the verge of significance.

One of the principal aims of the present investigation was to study the relative sensitivity to increased temperature, and this has, as mentioned above, been measured by the ratio of the survivals in 30°C and in 25°C, p_{30}/p_{25}. The results for the total material are shown in Table 3. All of the 12 comparisons between larvae from homozygotes and larvae from corresponding hybrids show superiority for the hybrids; this superiority is significant in ten of the cases. It is noteworthy that larvae from k, which in several cases were superior to their hybrids when developing in 25°C, show the most conspicuous relative decreases when comparing the survivals in 30°C and 25°C. In the case of larvae from stock flies, those from E and K are significantly inferior to their corresponding hybrids, while those from T are significantly inferior to only one of its hybrids. The comparison of larvae from stock flies with those from the corresponding homozygotes show that larvae from e are significantly superior to those from E.

Condensed survey of the results of the present experiments: Three types of tests are studied: (1) egg production, (2) the proportion p_{25} of larvae reaching the adult stage in 25°C, and (3) the relative decrease in larval survival measured by the ratio, p_{30}/p_{25}, of larval survival in 30°C to that in 25°C. Each of the three homozygotes was compared with the four hybrids into which they entered as parents, making 36 tests. Likewise, there were 36 tests for individuals from the stocks. Finally, the homozygotes were compared with the corresponding stock flies, making nine more tests. Table 4 summarizes the results. Though not all tests are independent (see first paragraph of the present section), it is clearly seen that in the great majority of tests, the homozygotes are significantly inferior to the corresponding hybrids. The same is true with regard to the comparisons between stock flies and their hybrids. The comparisons between homozygotes and stock flies give no clear picture.

To these results should be added that, with regard to egg laying capacity, pure flies (including homozygotes and stock animals) varied more within runs than did hybrids.

DISCUSSION

The characters studied in the present investigation, *viz.* egg laying capacity and larval survival, are certaintly of importance from a selectional and evolutionary point of view. The flies used in the experiments all emanated from stocks which for a very long time were kept at room temperature in ordinary stock

bottles. Mass propagation of the stocks was used, i.e., with intervals of between 15 and 20 days the content of the bottles were shaken over into fresh bottles. The flies produce usually heavy numbers of larvae, and larval competition within the bottles was probably as strong as under natural conditions. The number of flies shaken into fresh bottles was never counted but may be estimated to be around 200 of each sex. The relative smallness of this number may induce some degree of inbreeding (remember the low egg production of flies from the E stock), but otherwise the structure of the stock bottle populations may be considered to be rather similar to that of natural populations.

Concerning the fitness of wild Mendelian populations, the discussion mainly concerns two hypotheses, often called the "classical" and the "balance" hypotheses (see DOBZHANSKY 1959). In a study of the effects of low dose irradiation on *Drosophila melanogaster*, WALLACE (1958) gives a diagrammatic representation of the genetic content of an average individual according to the two hypotheses. The chief difference, as visualized in the diagram, is as follows. According to the classical hypothesis there is a large proportion of dominant homozygous loci, few heterozygous loci, and a limited number of alleles per locus; while, according to the balance hypothesis, there are few homozygous loci, many heterozygous loci, and a large number of alleles per locus. It is known (SPASSKY, SPASSKY, LEVENE and DOBZHANSKY 1958) that a large amount of genetic variability may be released through recombination, though this amount may differ between different chromosomes. It seems, therefore, inevitable to conclude that, at least within the species studied, the individuals have large numbers of heterozygous loci. This does not, however, in itself invalidate the essentials of the classical hypothesis. If one supposes that there is a high frequency of spontaneous mutations—probably much higher than hitherto assumed—and that each individual mutant gene has only a very low detrimental effect in heterozygous condition, one would, within the population's chromosomes, find a large number of long-lived mutant genes. But such a situation would not prevent the importance of one of the most characteristic attributes of the classical hypothesis, expressed by MULLER (1956) in the sentence ". . . the exceptional cases of the heterozygote being superior are probably represented for the most part . . . by adaptations that have not yet stood the test of geological time". According to this sentence, one has to postulate that homozygotes for genes which have stood the test of geological time, have received a homeostatic plasticity in magnitude equal to or surpassing that of heterozygotes in their capacity to withstand the stresses of changed environmental conditions.

The occurrence of hybrid superiority was known in pre-Mendelian time, and was considered in genetic analysis independently by SHULL and by EAST as early as 1908. In spite of this there is still no unanimity about its causes. (Hybrid superiority is known both from selfing plants and from outbreeding species. The discussion here will be restricted to the latter category.) The classical hypothesis is bound to the assumption that the average viability of individuals which are heterozygous for a specific locus never, or only in exceptional cases, exceeds the average viability of both of the two homozygotes. This is, thus, understood to

mean that hybrid superiority practically always depends on the interaction of nonallelic genes. The balance hypothesis is, in contradistinction to this, bound to the assumption of an effect *per se* of heterozygosity, that is to say that the average viability of individuals which are heterozygous for a specific locus may be superior to the average viability of both of the two homozygotes. This assumption has several names; here the word *overdominance*, proposed by HULL (1945) will be used. It should be stressed that adherents of the overdominance assumption certainly neither deny the presence of many instances where hybrid superiority is due to the covering effect of nonallelic dominant genes, nor do they pretend that all single gene heterozygotes show overdominance. From the point of view of evolution and of population dynamics one may, therefore, formulate the two opinions by saying that those who deny the truth of the balance hypothesis think that overdominance is at the very most of insignificant importance; whereas adherents of the balance hypothesis think that overdominance—though not the sole agent in provoking hybrid superiority—is common enough to play a profound and widespread rôle.

If there is hybrid superiority *within* a population, it is plausible to imagine that selection acts in favour of heterozygotes and so keeps their frequency on a high level. But when hybrids *between* unrelated populations, natural or artificial, show an increased viability in comparison with the parental populations—as has been proved to be common in several species of Drosophila—then it can hardly be a question of selection. In a series of studies VETUKHIV (1953, 1954, 1956, 1957) showed this to be the case for larval survival in *Drosophila pseudoobscura*, *willistoni*, and *paulistorum*, and for egg laying capacity and for longevity in *pseudoobscura*. Since the intercrossed populations were isolated from each other, he concluded that selection could not have been active, but that his results were in agreement with the assumption of an heterozygosity *per se* effect. Likewise, BRNCIC (1954) who worked with *pseudoobscura* found that the survival rate—i.e., the proportion of wild type flies after certain crosses involving marker chromosomes—was higher for population hybrids than for the parental populations.

NICOLETTI and SOLIMA (1959) found that, when starting each of five populations from 30 females and 30 males, and then comparing the number of flies after 60 days, the superior population was one which originated from a cross in which females and males descended from different populations. The three populations E, K, T, of the present investigation have been mass cultured at room temperature in our laboratory for several generations, ranging from about 200 (population K) to 600 or more generations (population E). "Room temperature" is usually between 18°C and 20°C; in summer time it may rise to about 25°C. A room temperature of 30°C, if it ever has occurred, is of very short duration and can certainly be excluded as a selectional stimulus. In spite of this, such a physiologically complicated character as relative resistance in larval survival was in most cases more marked for stock hybrids than for the parental stocks.

The superiority of hybrids between homozygotes over their parental forms is probably due to similar causes as that of hybrids between different populations. But the high level of viability of the former has not the same weight as the latter

when discussing the *per se* effect of heterozygosity. MAYNARD SMITH, CLARKE and HOLLINGSWORTH (1955) working with *Drosophila subobscura* found, among other things, that hybrids between two inbred lines descending from different populations had a more superior longevity than the parental inbred lines. BONNIER, JONSSON and RAMEL (1959) showed that the time span from egg hatching to eclosion was, in *Drosophila melanogaster*, shorter for larvae which were heterozygous for the two long autosomes than for those who were homozygous for these chromosmes.

In reviewing the subject MATHER (1955) expresses doubts against the assumption of an effect of heterozygosity itself. He says: "Even . . . in outbreeding species, like Drosophila, where heterozygotes characterstically show heterosis, its level is not always proportional to the degree of heterozygosity." This is true if one emphasizes the word *always* in this sentence. But when STRAUS crossed flies of *Drosophila melanogaster* from one population with flies of an inbred line of another population and then ordered the results according to the 27 possible combinations of hybridity and nonhybridity in the three pairs of major chromosomes, he got the following results for daily egg yield (arranged after an article by GOWEN 1952): homozygosity in all three pairs (eight combinations) 38.2 eggs; homozygosity in two and heterozygosity in one pair (12 combinations) 51.5 eggs; homozygosity in one and heterozygosity in two pairs (six combinations) 62.6 eggs; and heterozygosity in all three pairs (one combination) 76.9 eggs. In the study mentioned above, NICOLETTI and SOLIMA (1959) found a correlation between degree of heterozygosity and rate of growth of the different populations; there was, however, one exception: the least heterozygous population showed, at least to begin with, the fastest growth in number of individuals. This population was started from flies which had been inbred for 400 generations. (It is known that inbreeding not always is a reliable method for producing homozygosity; it would be interesting to know what would happen if real homozygous strains, by aid of an inversion marker method, were derived from this inbred population.)

MATHER (1955) points to the fact that: "the observation of overdominance has often been claimed and seldom, if ever, proved in relation to hybrid vigour, for it is not easy to distinguish . . . from interaction between nonallelic genes". In a recent paper, MUKAI and BURDICK (1959) reported on the viability of homozygous populations of *Drosophila melanogaster* into which they had introduced a single second chromosome lethal. The experiment is very interesting from the point of view of persistency of a lethal gene, as they find a high equilibrium frequency which is independent of genetic background and starting frequency. It is also possible that their case is an example of overdominance, even if one hesitates to say that they have proved this to be true. It seems uncertain that they, by using a very contracted technique for the process of homozygotization, really have developed homozygosis in chromosomes one and two. (Judging from a paper by BURDICK and MUKAI (1958) the authors seem to be aware of this.) Their method for determining the frequency of the lethal seems, from the description of their technique, to be open to question. Moreover, the second chromosome with

the lethal originated from the same population as that from which the homozygous population was produced. But the second chromosome, containing the lethal, must nevertheless, have differed from the second chromosome of the homozygous population with respect to several genes, apart from the lethal itself. These genes, though without necessarily directly interacting with the lethal, may interact *inter se* and may so contribute to the high viability of heterozygotes for the lethal. The fact that the frequencies of the lethal, observed during a number of generations, fit well with an expected curve, calculated from the assumption of a single gene pair effect, does not exclude the possibility of constructing a more or less similar curve on the basis of multigene effects.

It will perhaps remain impossible to give, by genetic analysis, a definite answer to the question of whether or not overdominance can occur in an outbreeding species. One may, however, approximate "one gene" by a short chromosome segment. This was done by BONNIER, JONSSON and RAMEL (1959) by making females of *Drosophila melanogaster* wild type isogenous for the whole length of the X chromosome except for a segment including the *w* locus and being of a maximum length of 4.7 map units. The time from egg hatching to eclosion was compared for female larvae which in the X were either +/+ or heterozygous for + and one *w* allele. It was found that the homozygotes +/+ were superior, but it was emphasized that a different result might be found if a similar experiment were to be performed with a short autosomal segment.

It ought to be stressed once more that certainly not all heterozygous loci give overdominance effects. Several examples could be given. Suffice here to mention the studies on asymmetries of sternopleural bristle numbers in *Drosophila melanogaster* by THODAY (1955). He had shown that asymmetry was inversely correlated with viability. X chromosomes were derived from populations that had been adapted to two different environments, and it was found that whereas intrapopulational hybrids had a low degree of asymmetry in their own as well as in the foreign environment, the interpopulational hybrids had a high degree of asymmetry in both environments.

HAGBERG (1953) who has made a very thorough investigation on hybrid superiority (though mostly on selfing plants) emphasizes two different ways by which overdominance may be produced: "(1) the two alleles function as complements . . . together they result in a better effect in the heterozygote than each of them in a homozygous state are able to produce. (2) One of the alleles may be without effect, may even be a deficiency—the other allele has an optimal effect when in single dose and the homozygous state is an overdose". (With regard to the second possibility he refers also to FISHER 1918). But MUKAI and BURDICK (1959) point out that if one gene controls only one chemical reaction or produces only one enzyme, the first of HAGBERG's assumptions could not be valid. This remark seems, however, not to be essential in such cases where one of the genes is neomorphic to its allele. HALDANE (1955) refers to some cases in which it could be possible to explain overdominance on biochemical grounds. In one of these (the case of lozenge studied by CHOVNICK and FOX), it is known that the two alleles are pseudoalleles. But as one can hardly be sure that any pair of alleles,

will not with refined techniques turn out to be pseudoalleles, one may ask if this, in respect to the problem of overdominance, is of critical importance. One of the methods which PONTECORVO (1958) describes for selecting mitotic crossovers in *Aspergillus nidulans* is based on the fact that germinating conidia having certain combinations of requirements survive longer than those of strains having only one of these. He mentions, for example, that on a medium lacking adenine and biotin, double adenineless-biotinless mutants survive much longer than biotinless ones. Even if this may seem to be a peculiar event, it is a fact, and because of this, one has reason to agree with HAGBERG (1953) who said: "Complementary genes give together an effect which each of them alone cannot produce. It seems reasonable to assume that two alleles may be complementary in a similar manner, and there is nothing astounding or 'unnatural' in the phenomenon of superdominance. Rather, it would be 'unnatural' to assume that it could never occur".

CONCLUSIONS

Many cases of hybrid superiority are certainly due to interactions of nonallelic genes and many heterozygotes are certainly nonoverdominant. It is possible that every case of hybrid superiority could be explained without accepting overdominance. But such explanations would in many important instances, e.g. the superiority of hybrids between nonrelated wild type populations, probably be of quite a formal kind, involving complicated accessory assumptions and constructions of interactions between nonallelic genes. The single assumption of overdominance seems to suffice for making many accessory assumptions unnecessary. As it is known that the combination of inferior genes may make a superior product (see above about *Aspergillus nidulans*), one seems to be entitled to conclude that, as long as there is no explicit biochemical proof to the contrary, the assumption of overdominance—sometimes caused by stimulation of two inferior genes, so as to make their sum superior; sometimes caused by one of the homozygotes being an overdose—is the simplest and most probable tool that nature has evolved within many important fields of the dynamics of natural populations.

SUMMARY

1. Three unrelated wild type stock populations of *Drosophila melanogaster* were used in the present study. From each of these stock populations one homozygous population was derived. Viability experiments were made with flies taken directly from the stocks ("pure" flies) and with the six possible F_1 hybrids between them. The same types of experiments were made with pure homozygous flies and with the six possible F_1 hybrids between them.

2. The viability studies included three characters: (1) egg laying capacity, (2) capacity of larvae to survive to the adult stage in 25°C; this character was measured by p_{25} = the proportion of freshly hatched larvae which reached eclosion, (3) larvae were also reared in 30°C, and their capacity to survive was measured by the corresponding proportion p_{30}. The third character studied was, however, not p_{30} itself but the relative decrease in survival rate when comparing

survival in 30°C (strong stress) to survival in 25°C (no stress). This character was measured by the ratio p_{30}/p_{25}.

3. The hybrids were the superior ones in the great majority of cases and for all three characters.

4. After a discussion of the results and after reviewing similar results from the literature, emphasis is laid on the difficulties to explain the superiority of hybrids between unrelated populations as caused by selection. It is concluded that the single assumption of overdominance will make many accessory assumptions unnecessary. As long as there is no biochemical proof to the contrary, the assumption of overdominance seems to be the simplest and the most probable tool that nature has evolved within many important fields of natural population dynamics.

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THE GENETIC BASIS OF NATURAL VARIATION. III. DEVELOPMENTAL LABILITY AND EVOLUTIONARY POTENTIAL[1,2]

ROGER D. MILKMAN[3]

Department of Zoology, The University of Michigan, Ann Arbor

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GENES change and organisms evolve. The connection between mutation and evolution must be understood in terms of both gene action and the existing organism. This paper, following two which presented a fairly classical analysis of a polygenic system, is concerned with the developmental process affected by these polygenes acting in concert.

It has been shown (MILKMAN 1960a,b,c) that a group of naturally important genes, the *crossveinless complex*, interfere with the formation of the posterior crossvein in *Drosophila melanogaster*. This system was first studied by WADDINGTON (1953a), and later by MILKMAN (1956, 1958), MOHLER (1958) and BATEMAN (1956, 1959). Similar genetic systems have been studied from various points of view by LERNER (1954), SOBELS (1952) and DUBININ (1948). BARIGOZZI, CASTIGLIONI and DI PASQUALE (1960) have made an extensive investigation of polygenic systems and developmental processes concerned with the formation of melanotic tumors in Drosophila.

All these cases are similar in that they deal with polygenic systems of apparent natural importance and with phenotypes whose appearance is quite sensitive to environmental factors. The findings of these workers heighten the interest in understanding labile processes in development: first, because these are the events of development most subject to controlled experimental attack, and second, because they are found to be associated with genes important in nature. The basis of this association seems rather simple, and it will be taken up in the discussion of the present findings.

MATERIALS AND METHODS

The posterior crossvein of *D. melanogaster* makes its appearance late in the first day after puparium formation (WADDINGTON 1941). Not unexpectedly, this is when the developing animal is sensitive to disturbance of posterior crossvein formation (WADDINGTON 1953a). The experiments to be presented here were performed mostly with animals raised at 23°C, since at this temperature the

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[3] Present address: Zoology Department, Syracuse University, Syracuse 10, N. Y.

Oregon R strain reaches its peak sensitivity to disturbance of crossvein formation 24 hours after puparium formation. The animals were collected during the hour or so in which they had formed the puparium but before they had tanned, and the treatments were carried out on the following day at approximately the same time.

The puparia adhered to the walls of the empty shell vials in which they were placed (generally 30 per vial were used). The vials were plugged with cotton which was kept moist until the flies emerged, in order to prevent desiccation. At the appropriate time, a vial was removed from the incubator, fitted with a holder, and submerged in a Precision water bath with temperature maintained within several hundredths of a degree C. The holder consisted of a u-shaped glass tube terminating in a rubber stopper. This permitted complete submersion of the vial and air exchange with the outside. The free end of the glass tube was held against the side of the bath by a rubber belt. The inner wall of the shell vial reached 40°C (when that was the bath temperature) from room temperature in five minutes, according to thermocouple measurements, and this agreed with calculations based on experimental results. This paper is concerned mainly with treatments in the range 39.5°–41.5°C.

The period of sensitivity to disturbance of posterior crossvein formation extends for several hours, and its length depends upon the dosage. Response is measured in terms of the average crossvein defect (= rating) of the sample. The units are twelfths of crossvein missing. A fly with 3/4 of its posterior crossveins gone (9/12) is rated "9", and a normal fly is "0". For further discussion of this rating system, see MILKMAN (1960b).

A number of variables markedly affect the results. Most of these can be controlled rather well, but the rate of aging of pupae is an exception. Although it is generally the case that animals aged at 23°C will be maximally sensitive at 24 hours after puparium formation, it is still considered necessary to run the treatments in triplets; one at 23 hours, one at 24, and one at 25. Thus one can be sure of hitting the peak sensitivity period.

EXPERIMENTAL RESULTS

The results will be grouped into five categories: response *vs.* age; response *vs.* dosage; temperature coefficient of treatment; response *vs.* genotype; and a brief summary of related data.

1. *Response* vs. *age:* The period of sensitivity to the heat treatments discussed here lasts for several hours. Figure 1 shows several age-response curves. At peak sensitivity, all the treated animals will respond to a strong heat shock (e.g. 40 minutes at 40.5°C or 20 minutes at 41.5°C, including warmup time). Before and after the time of peak sensitivity, the degree of individual response drops off, and so does the proportion of animals responding. This shows that the sensitive period graphed is fairly representative of the individual, rather than being only a summation of a population of short sensitive periods at varying ages. Table 1 is representative of the experimental results.

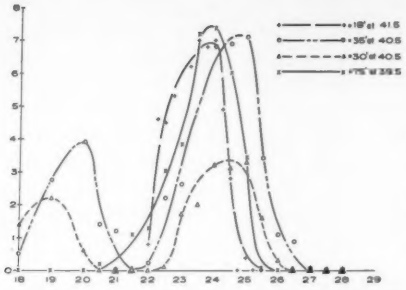

FIGURE 1.—Age-response curves for Oregon R females raised at 23°C. Ordinate: average crossvein defect rating. Abscissa: hours after puparium formation.

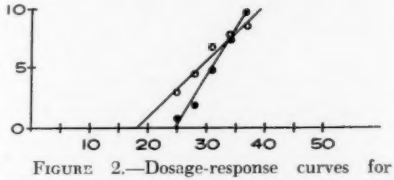

FIGURE 2.—Dosage-response curves for Oregon R females (open circles) and males (solid circles) raised at 25°C. Ordinate: average crossvein defect rating. Abscissa: time in minutes at 40.5°C.

TABLE 1

Age-response data

	Rating							
	18 min 41.5°C		35 min 40.5°C		30 min 40.5°C		75 min 39.5°C	
Age	Males	Females	Males	Females	Males	Females	Males	Females
18	–	–	0.4	0.5	0	1.4	0	0
19	–	–	1.5	2.7	0	2.2	0	0
20	–	–	1.5	3.9	0	0.5	0	0
20½	–	–	2.3	1.4	0.6	0	0.1	0.2
21	–	–	0.4	1.2	0.7	0	0	0
21½	–	–	0.3	0	0	0	0.6	1.1
22	0	0.8	0	0.2	0	0	2.8	1.3
22¼	0.6	4.6	–	–	–	–	–	–
22½	1.9	4.5	0.8	2.2	0	0.1	3.2	3.0
22¾	3.5	5.3	–	–	–	–	–	–
23	4.0	7.0	1.5	2.6	0.8	0	4.3	3.8
23¼	1.6	6.2	–	–	–	–	–	–
23½	1.6	7.0	1.4	2.0	0	1.7	5.8	7.4
23¾	2.0	6.8	–	–	–	–	–	–
24	–	7.0	4.0	6.8	0	3.2	6.7	7.2
24¼	2.0	4.9	–	–	–	–	–	–
24½	0.6	2.8	5.2	6.9	0.8	3.1	5.8	6.0
24¾	0.3	0	–	–	–	–	–	–
25	1.0	0.4	4.4	7.1	0	3.3	4.7	3.4
25¼	0	0	–	–	–	–	–	–
25½	0	0	1.0	3.4	0	1.6	0	0.1
26	–	–	0.2	1.1	0	0.3	0	0
26½	–	–	0.9	0.9	0	0	0	0
27	–	–	0	0	0	0	0	0
28	–	–	0	0	0	0	0	0

Another way of demonstrating the same point is by the use of summation experiments. Animals may be treated subliminally at 24 hours and at another time, either before or after, to show the length of the individual's sensitive period. Summation of these two treatments produces crossvein defects. Such experiments also indicate that the individual sensitive period lasts several hours; however, these experiments are valid only if it can be clearly shown that the first treatment does not temporarily arrest development, causing the second treatment to be given at a developmental age very close to that of the first. Detailed presentation of such experiments will be deferred until later (MILKMAN, 1961b).

2. *Response* vs. *dosage:* It should be noted that the results in this section concern pupae aged at 25°C, not 23°C. This experiment was designed to complement a set of crosses done at 25°C. At 40.5°C, treatments up to about 20-minutes' duration at peak sensitivity produce no crossvein defects. Longer treatments produce defects, and these increase in frequency and extent with increasing treatment duration. Males and females have different dosage-response relationships, as can be seen in Figure 2, in which the average crossvein defect rating is plotted against duration of heat shock. It will be seen that response rises linearly with duration and that the males start responding later but eventually catch up with the females.

It would appear that the heat treatment results in the progressive destruction of some factor necessary to posterior crossvein formation. The existence of a threshold implies that the developing pupa has more of this factor than it actually needs and that the destruction of a certain amount of this factor does not result in crossvein defects. When the "reserve" is exhausted, however, further destruction of the factor does bring about visible results. Moreover, it would appear that males and females have different reserve amounts (since they have different thresholds), and the rate of destruction appears different in each of the sexes.

To carry this reasoning to its logical conclusion, one might estimate the amount of "reserve" crossvein-making ability in each sex by extrapolating these apparently linear curves back to the start of the treatment. This would be when the pupae had reached 40.5°C, or about five minutes after immersion in the bath. Figure 3 illustrates this extrapolation; the method of least squares was used; values of −8.5 and −19.5 were found for females and males, respectively. Measured in units corresponding to 12ths of the normal posterior crossveins, these values indicate that an Oregon R female raised at 25°C has enough of this factor in reserve to make a partial set of crossveins (8.5/12). A male has enough to make about 1 2/3 sets of crossveins (19.5/12). Both these values are in addition to the amount actually used.

These estimates of the reserve crossvein-making ability of Oregon R flies agree fairly well with estimates based on the results of genetic crosses (MILKMAN 1960b). In the earlier experiment, Oregon R flies were crossed to a true-breeding polygenic crossveinless *(cve)* strain and backcrossed twice to the *cve* parents. The additive action of the *cve* genes enables one to calculate the reserve crossvein-making ability of the Oregon R flies. On this basis, females were rated −13 and males −19. These two independent approaches, then, give estimates in fairly

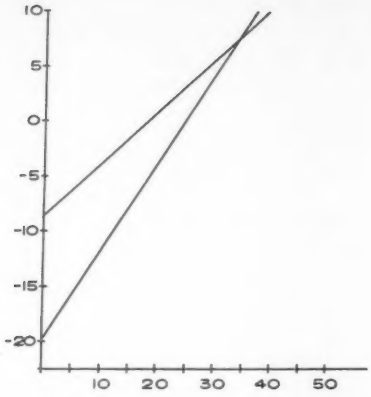

FIGURE 3.—Extrapolation of Figure 2 back to zero time. Ordinate: crossvein defect rating (minus values represent reserve crossvein making ability). Abscissa: time in minutes at 40.5°C.

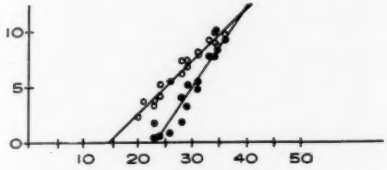

FIGURE 4.—Dosage-response curves for Oregon R females (open circles) and males (solid circles) raised at 23°C. Data converted to equivalent times at 40.5°C assuming $Q_1=2.3$. Ordinate: crossvein defect rating. Abscissa: time in minutes at 40.5°C.

close agreement of the reserve crossvein-making ability of Oregon R flies: for females, −8.5 and −13; for males, −19.5 and −19.

This character depends upon the temperature at which the pupae are aged. From 18° to 28°C, the higher the aging temperature, the more reserve crossvein-making ability is indicated. For females and males, respectively, the values at 23°C are −7 and −17, and at 28°C, −15 and −25. Correlated with this effect of pupal temperature on phenocopy sensitivity are the effects of pupal temperature on the expression of the *cve* genes. Wild strains show a higher incidence of *cve* phenotypes at 18° than at 25°, *cve*/Ore R heterozygotes show the *cve* phenotype with greater penetrance and expressivity at 18°C than at 25°C; and *cve* strains in general (five have been examined for this trait) show greater defects at 18°C than at higher temperatures in the physiological range. An example of the last case is this: in a true-breeding *cve* strain isolated from flies collected in Mexico, the following average crossvein defect ratings were obtained for males and females, respectively, at 28.5°C, 4.0 and 7.6; at 25°C, 8.2 and 9.1; and at 18°C, 11.6 and 10.0. The temperature has its effect during the first day of pupal life.

3. *Temperature coefficient of the treatment:* It is now proper to inquire as to the nature of this factor which we have up to now called "crossvein-making ability", surely an overstatement of its importance. Because of the temperature at which it is destroyed, and because almost everything done in living cells is done by enzymes, one is first impelled to ask whether this factor is not a protein, or a function of a protein. This question has one relatively easy approach because of a distinctive property of proteins applicable to the phenomenon under study:

the denaturation of proteins has a temperature coefficient far and away greater than any other chemical reaction of biological significance which proceeds in this range of temperatures; and in fact measurement of the temperature coefficient of the destruction of "crossvein-making ability" points clearly to protein denaturation.

A brief summary of the methodology used here will be of value. First, it was found that a half degree makes a great deal of difference in the treatment duration needed to produce a given response. Second, a series of treatments at various temperatures and durations was set up. All treatments producing quantitatively similar responses were compared. In the range from 39.5°C to 41.5°, a Q_1 of 2.3 was found to fit the data well. This corresponds to a Q_{10} of over 4000. The value was uniform for half-degree intervals throughout this range. Finally, treatments at two temperatures within the range were found to summate as the temperature coefficient would lead one to predict. Thus one seems to be dealing with a single process: the denaturation of a protein which is necessary to posterior crossvein formation. Our present working hypothesis assigns this role to a protein which will be called crossvein protein "A". Its quantity, role, and physicochemical properties must make its destruction the limiting factor in production of crossvein defects by treatments in the range from 39.5°C to 41.5°C. On the other hand, two distinct additional temperature ranges for disturbance of posterior crossvein formation give evidence of additional susceptible processes.

Estimation of the Q_1 as 2.3 was independent of dosage-response relationships, since treatments producing quantitatively similar results were compared. All data collected from the various treatments could now be converted to equivalent times at 40.5°C. As usual, the male and female dose-response curves were different, but both were linear. Comparison of these curves (Figure 4) with Figure 2 will show different intercepts resulting from the fact that the data graphed in Figure 2 were obtained from animals aged at 25°C, while the present experiment employed animals raised at 23°C. Extrapolation of these curves back to the beginning of the treatment (Figure 5) shows the reserve values for animals raised at 23°C.

Figure 6 provides a test of the validity of three elements of the system: (1) linearity of the dosage-response relationship, (2) reserve crossvein-making-ability values, and (3) uniform, high temperature coefficient ($Q_1 = 2.3$) from 39.5°C to 41.5°C. If any of these should be markedly wrong, the points in Figure 6 should not fall on the lines. In fact, the data do support the hypothesis. Each line represents the rate of denaturation at a given temperature. Its slope is calculated from the slope in Figures 4 and 5 by the use of the $Q_1 = 2.3$. Its point of departure is that found for the sex in question in Figure 5. The picture, then, is consistent with a constant-rate process having a Q_1 equal to 2.3 and having a starting point dependent upon sex and, apparently, aging temperature.

4. *Response* vs. *genotype:* Needless to say, a process affected by so many environmental factors could hardly be indifferent to variations in genotype. Three kinds of variation will be discussed: introduction of *cve* genes (MILKMAN 1960b), other wild-type strains, and introduction of the marker gene crossveinless-*c*

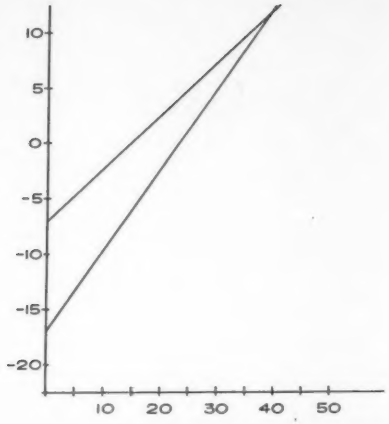

FIGURE 5.—Extrapolation of Figure 4 back to zero time. Ordinate: crossvein defect rating (minus values represent reserve crossvein making ability). Abscissa: time in minutes at 40.5°C.

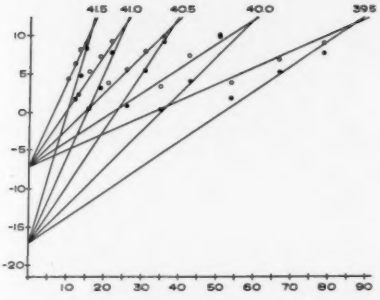

FIGURE 6.—Dosage-response curves for Oregon R females (open circles) and males (solid circles) at temperatures indicated. Same data as figure 4. Ordinate: crossvein defect rating (minus values represent reserve crossvein-making ability). Abscissa: minutes at indicated temperature.

(*cv-c;* MILKMAN 1960c). These experiments also deal with pupae raised at 25°C, not 23°C, so that the ages are not equivalent to those discussed in parts 2 and 3.

The *cve* polygenes were selected partially on the basis of their ability to increase crossvein defect production by heat shock. When they were finally assembled into a true-breeding crossveinless strain, this strain was crossed to Ore R, and the F_1 was tested for crossveinless phenocopy susceptibility. As expected, it took far less heat shock to produce crossvein defects. The *cve* genes, acting on the quantitative character "crossvein-making ability", had ostensibly had an effect, direct or indirect on Protein A. Figure 7 shows an age-response curve for *cve*/Ore R hybrids (treatment duration:20 minutes). In addition, the effect of raising the pupae at 18°C rather than 25°C is shown: *cve*/Ore R flies show a low frequency of crossvein defects when raised at 25°C, and this frequency is increased markedly when the flies are raised at 18°C. Specifically, four-hour periods at 18°C are effective with midpoints at the times shown on the graph. The bimodality of this phenocopy curve is the most striking example of a usual observation. It should be noted that some other *cve* genes, assembled in true-breeding strains from wild parents, do not influence phenocopy sensitivity at 40.5°C.

Several other wild-type strains were tested. Figure 8 shows age-response curves (30 minutes at 40.5°C) for Oregon R and for two mass-mated strains, Vandeventer Flat and XSA-7. The XSA-7 is related to the parental strain of *cve*. The Vandeventer Flat flies, incidentally, showed a marked tendency towards all-or-none phenocopying of a given wing. The bimodality of the curve is also clear.

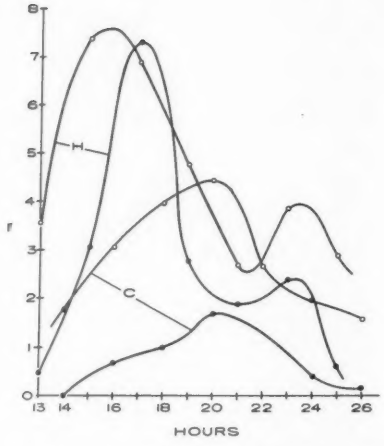

FIGURE 7.—Age-response curve for *cve*/Ore R females (open circles) and males (solid circles). "H"—20 minutes at 40.5°C. "C"—4 hours at 18°C. Ordinate: crossvein defect rating. Abscissa: hours after puparium formation at 25°C.

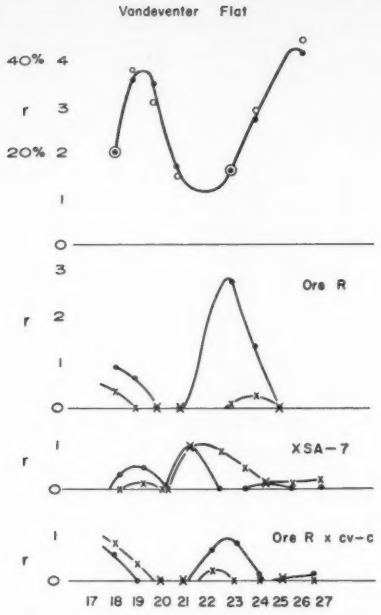

FIGURE 8.—Age-response curves for other strains treated at 40.5°C for 30 minutes. Ordinate: crossvein defect rating. Abscissa: hours after puparium formation.

Finally, Oregon R flies were crossed to flies homozygous for *cv-c*, a simple recessive causing the near absence of the posterior crossveins. The *cv-c* heterozygotes showed no increase in crossveinless phenocopy susceptibility. Although differences in genetic background might have compensated for the presence of *cv-c*, it is more likely that *cv-c* does not affect the concentration of protein "A", but rather affects the formation of the posterior crossveins in some other way.

5. *A brief summary of related data:* Outside of the 39.5°C–41.5°C range, different effects are observed. Some are spectacular. The results are summarized in Table 2. The remarkable temperature sensitivity of what must be various elements in the process of posterior crossvein formation is striking. Experiments designed to gain insight into the relationships of these various elements to one another and to the animal as a whole will be described and discussed in a later paper (MILKMAN 1961b). Suffice it to say for now, that control of the variables according to methods listed above permits essentially unfailing repeatability of these findings on Oregon R flies.

TABLE 2

Summary of effects of various temperatures on D. melanogaster *pupae 24 hours old (at 23°C)*

Temperature	Duration	Effects
42.5	8–10 minutes	Posterior crossvein defects; death
42.0	11–14	Posterior crossvein defects; death
41.5	12–20	*cve*
41.0	18–30	*cve*
40.5	25–45	*cve*
39.5	55–97	*cve*
39.0	90–153	*cve* (longer duration needed than expected on basis of $Q_1 = 2.3$)
38.8	2–3 hours	as 39.0
38.5	2–5	no effects except less than one percent *cve*
38.2	4	no marked effects
38.0	4–5	"38.0 complex"*
37.8	4	*cve* in males; rare in females; some "37.5 complex"†
37.5	4–5	*cve* "B"; "37.5 complex"; antagonizes effects of 39.5–41.5°†
37.2	4	*cve* "A" and "B" in females mostly some branching of posterior crossvein
37.0	4–6	*cve;* smudge on posterior crossvein; reverse dose effect‡
36.8	4	as 37.2
36.5	4–7½	*cve;* antagonizes 39.5–41.5 effects
36.2		
36.0	3–5	*cve;* reverse dose effect‡
35.5	3–7	no effect
35.0	5–7	no effect

* "38.0 complex" = rare posterior crossvein defects; frequent anterior crossvein defects; wings wavy, flimsy, and smaller; smudge on posterior crossvein; longitudinal veins "leaky" (little dots and streaks of vein material nearby); bristles short and deformed.

† "37.5 complex" = longitudinal veins 3 and 4 abnormally close; elliptical hole between L_3 and L_4; wings held out, small; anterior crossvein defective. Only anterior crossvein defects are generally seen in females.

‡ Reverse dose effect = after peak response is reached, increasing duration reduces response, often to none at all.

cve-cve "A" = missing segment near L_4; *cve* "B" = segment missing from center of crossvein.

DISCUSSION

At a certain stage in development, the developing pupa is making its posterior crossveins. At this time, and just before it, rather general environmental influences (heat shock, low temperature in the physiological range) can prevent perfect crossveins from being formed in flies of most genotypes tested. In addition to those reported in this study, 21 other strains of *D. melanogaster* (MILKMAN, 1961a) and two other species (*D. virilis* and *D. funebris*) respond to 40.5°C heat shock at 24 hours, or at a corresponding age, by failing to develop perfect posterior crossveins. Of all the developmental processes going on at this time, then, the formation of posterior crossveins is the most sensitive to temperature effects in certain ranges. On the other hand, at other high temperatures or in other strains, some vital process may be as sensitive to disruption, and death may precede

phenocopying. But, defining our conditions, we may characterize one or more elements of the process leading to posterior crossvein formation and thereby come to understand more fully the mediators of gene action.

From the dosage-response experiments presented in sections 2 and 3, we have arrived at two parameters: "reserve crossvein-making ability" and rate of destruction of this crossvein-making ability. The values for the first agree with those obtained by crossing a polygenic *cve* strain with Oregon R and backcrossing to *cve* (MILKMAN 1960b). Thus a range of phenotypes, perfect crossveins to no crossveins ("0" to "12"), has been extended beyond its standard-conditions visible range to run from fairly high phenocopy resistance to the inability to form posterior crossveins under any conditions observed ("−19.5" to "+12", or "8.5" to "+12"). The temperature coefficient measurements in the 39.5°–41.5°C range indicate that this crossvein-making ability is dependent upon a protein, which we call "protein A". Protein A is one element, then, of the process leading to posterior crossvein formation. Since the denaturation of protein "A" would be expected to follow an exponential decay curve (i.e., log concentration of Protein "A" should be linear with time) and since the destruction of crossvein-making ability is itself linear with time, it would appear that crossvein-making ability is a function of the log concentration of Protein "A".

Other possibilities exist, however, which might explain the empirical linearity of the destruction of crossveinmaking ability. Protein A has three attributes that can be measured relative to the amount of protein A needed to make a perfect set of posterior crossveins. The three measurables are: amount normally present; rate of denaturation at, say, 40.5°C; and temperature coefficient of the rate of denaturation. Any of these three might be affected by the various *cve* polygenes, and it should be stated explicitly that these effects might be direct or indirect in any of the three cases. In the first case, one might assume that concentration of a protein might only be affected indirectly by genes, that is, not by a change in its amino acid sequence. However, recent findings in various hemoglobin studies (cited in NEEL 1959) indicate that in heterozygotes abnormal hemoglobins do not necessarily appear in the same amounts as normal hemoglobins. Second, it is not difficult to see that the rate of protein denaturation as a given temperature depends on both the protein and the milieu. Finally, though one might be inclined to regard a denaturation temperature coefficient as a protein's own, come what may, the surrounding medium, by affecting the activation of energy of this process, automatically affects the temperature coefficient.

By the use of the Arrhenius equation (cited in FRUTON and SIMMONDS 1953), one can calculate the energy of activation of the denaturation of Protein A in the 24 hour old pupa.

$Ln\ K = \frac{A}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$, where K is equal to the rate at the higher temperature over the rate at the lower temperature; R is the gas constant and here is taken as 2.0; T_1 and T_2 are the two temperatures (absolute); and A is the unknown, activation energy. Here $K = Q_1 = 2.3$. Thus, $ln\ 2.3 = \frac{A}{2}\left(\frac{1}{313} - \frac{1}{314}\right)$ and $A =$

about 160 Kcal/mole, which is in the upper range of values for protein distribution. It may be pointed out that this *in vivo* measurement is one of the more precise of its kind, due to the fortunate fact that in the range considered, Protein A is unaccompanied in its degree of lability by any other factor in posterior crossvein formation, since its destruction limits crossvein formation.

Turning now to the effects of the *cve* genes on phenocopy sensitivity, one is struck by the broadening of the age-response curve. This is relevant to a favorite question of WADDINGTON's (1953b, 1957). The question has often taken two forms: How can natural selection account for the prenatal thickening of a baby's soles? How can an ostrich's protective calluses begin forming in the egg as a response to selection?

A glance at the *cve* genes' effects over time gives a good illustration of a general answer: when one selects for a trait, one selects for the developmental process leading to that trait; and when one selects for certain characteristics in a developmental process at one time, it is hard to conceive of not bringing along the same characteristics at other times.

The *cve* genes were selected for their increase of heat-induced crossvein defects, when the heat shock was given at 21–24 hours. Now these same genes can be shown to have the same effect at 15 hours. Clearly, it is not correct to visualize each time-transect as independent of its neighbors at this level of organization.

The relationship between developmental lability and evolutionary potential may now be discussed. It has long been known from the phenocopy work of GOLDSCHMIDT (1955) and many others that certain processes are salient in their sensitivity to rather general unfavorable influences at specific times in development. The time of the shock and the particular sensitivity of the process lend specificity to the treatment. The results described above add more detail to the picture but do not change it markedly. The morphological disturbances produced are evidence of comparative lability of some developmental process.

Since it has been shown (MILKMAN 1960a) that common genes affect the lability of crossvein development, on an absolute scale and relative to other contemporary developmental processes in the fly, the connection between developmental lability and evolutionary potential is clear: genes common in nature affect the ability of each fly to produce crossveins; environmental variation is also of great impact. In short, the situation is ideal for the evolution of posterior crossvein defects, should they prove of value. Many different combinations of *cve* genes on many different backgrounds all have the possibility of leading the way to this morphological change. Of course, nature has not picked up this option. Otherwise *Drosophila melanogaster* would not have posterior crossveins. We must be content with studying evolutionary processes that have not gone all the way.

Now, why are so many genes involved with posterior crossvein formation? To answer this question, we may begin with a statement on the specificity of the gene-phenotype relationship. This depends upon two things: the chemical product of the gene and the vast array of developmental processes into which this product is released. In other words, to explain the relationship between a certain

gene and a certain phenotype, we must look hard at both. A particularly sensitive developmental process will respond to a particularly large number of gene changes, thus associating many genes with it, in our view.

Again, the posterior crossvein and the *cve* genes provide a good case in point, for we can reconstruct what must happen routinely in evolution. As evolution takes place, the stabilization of one labile process doubtless leaves another vulnerable (since our little information on the subject almost always shows some processes to be more sensitive than their contemporaries). This newly vulnerable process is now a particular sensitive indicator of genetic variation. We mimic this by enhancing the already marked sensitivity of crossvein formation through the use of heat shock. Animals pushed closer to the brink of crossvein defects now reveal the presence of *cve* genes that wouldn't have mattered (and that we would not have called *cve* genes) before. Indeed, this method enabled WADDINGTON first to assemble a group of *cve* genes from a population in which all flies had perfect crossveins.Thus the *cve* genes were named for their effects on an intentionally weakened process. What other things they do are, without a doubt, of far greater importance.

We can now see a mechanism of genetic recruitment, a means of speeding evolution, once it has begun. Once a developmental process loses some of its buffering, genes with previously negligible effects on this process join the team and speed the change. In this way natural selection capitalizes on existing genetic variation, providing grist for its own mill.

This mechanism, can give greater meaning to the often apparently purposeless genetic variability of populations, and it can provide a more concrete example of the importance of environmental variation. A hypothetical example illustrates the working of these factors: Suppose an ordinary population with a moderately well-buffered trait produces, under the rare combination of a certain extreme environment and a particular combination of genes, a variant in this trait which is highly adaptive. These genes are selected for. While they do not usually produce the new trait, they "unbuffer" the old one, so that the new trait can be produced in a wider range of environments and a wider range of genotypes. Finally, enough genes are recruited to stabilize the new trait in all environments generally encountered. This mechanism may help our understanding of several striking cases of parallel evolution, notably the evolution of mammals, which according to SIMPSON (1953) took place at least five separate times. It follows from recent observations (MILKMAN 1960a, 1961a; see also DUBININ 1948) that if all the *Drosophila melanogaster* in the world were divided into isolates of ten (five of each sex) and permitted to produce 10,000 progeny each, over 99 percent of the isolates would give rise to some *cve* flies, and perhaps half of them could evolve into strains breeding true for crossvein defects.

It is taken for granted that evolution could proceed far into the future on the basis of the genetic variation now present in most natural populations. The present discussion leads us to formulate three concepts of the relationship between mutation and evolution. Two are well-known, the third has perhaps not been

stated explicitly. First and simplest: a single mutation may lead to an adaptive phenotype at once. An example of this is the sickle cell gene in malaria country. This gene is maintained in populations until malaria is wiped out (NEEL 1959). Perhaps industrial melanism sometimes takes place through this simplest mechanism, even though not all of the melanic mutations are selected (KETTLEWELL 1955).

Second, a mutation may have to be followed by recombination leading to some co-adaptive set of characters. If the mutant gene has not disappeared, it may now be selected together with the other relevant genes. Third, a mechanism which may prove to be the most common. Here, new mutant genes would be incorporated into the gene pool only on the basis of their ability to mediate the normal functions of the organism better than the existing genes. Alternatively, they might improve the performance of certain functions when they are present in moderate frequencies (heterotic effects). Now, it is the difference between such new genes and their "older" alleles which suddenly becomes important in affecting some newly unbuffered developmental process. The "new" genes now take over. When they were present at 50 percent frequency, very few flies had many of the genes homozygous at once. At frequencies near 100 percent, they change the complexion of the whole population.

The experimental modification of development reveals unexpressed developmental potentialities and thereby evolutionary potentialities.

SUMMARY

1. Interference with posterior crossvein production in *D. melanogaster*, Oregon R strain, by means of exposure to temperatures ranging from 39.5°C to 41.5°C has been studied in terms of sensitive period, dosage *vs.* response, and temperature coefficient.

2. It is suggested that this phenocopy process is mediated by denaturation of a protein.

3. Reserve amounts of crossvein-making ability are estimated in terms of the amount needed to make perfect crossveins.

4. Results obtained with other strains of flies are presented.

5. The *cve* polygenes in this investigation increase phenocopy sensitivity at 40.5°C. Some other *cve* polygenes do not.

6. Results of treatments in the range from 35°C to 42.5°C are presented briefly.

7. The relationship between developmental lability and evolutionary potential is discussed.

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AUTOSOMAL NONDISJUNCTION IN MALES OF DROSOPHILA MELANOGASTER[1,2]

JUSTIN N. FROST[3]

Department of Zoology, University of California, Berkeley, California

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BECAUSE triploid females form large numbers of chromosomally unbalanced eggs it is possible to recover complementary types of unbalanced sperm. This technique revealed the occurrence of autosomal nondisjunction in males (STERN 1934; PONTECORVO 1940) and was also used by MULLER and PONTECORVO (1940) and PONTECORVO (1943) to recover sperm where radiation induced chromosome breaks caused one or more paternal chromosomes to be lost in the embryo. The same technique was employed for the present study of autosomal nondisjunction in males.

There were two reasons for undertaking such a study, the first of which was to discover how males differed from females with respect to interchromosomal effects of inversions on segregation. Heterozygous inversions in females are known to have interchromosomal effects on crossing over and segregation, and COOPER, ZIMMERING and KRIVSHENKO (1955) have suggested that the interchromosomal effect of inversions on the frequency of X chromosome nondisjunction is caused by non-homologous pairing between the sex chromosomes and autosomes. If similar interchromosomal effects on segregation occurred in males, where crossing over is absent, the influence of inversions on segregation could be studied apart from their influence on crossing over. It was also hoped that a simultaneous study of sex chromosomal nondisjunction and autosomal nondisjunction might serve to test for the occurrence of non-homologous pairing. The second reason for studying autosomal nondisjunction in males was to reconsider STERN's results. Over half the exceptions recovered by STERN required for their explanation the fertilization of a nondisjunctional egg (nullo II or nullo III) from a triploid female, by a nondisjunctional sperm (diplo II or diplo III) from a diploid male, and the number of exceptions recovered indicated that autosomal nondisjunction was fairly frequent.

In order to understand the type of interchromosomal effects on segregation that might be expected in males, it will be necessary to review briefly the present knowledge of such effects in females. In females with structurally heterozygous X chromosomes the frequency of primary nondisjunction is increased when a

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[3] Present address: Department of Biology, University of Oregon, Eugene, Oregon.

heterozygous inversion is present in an autosome (STURTEVANT 1944; COOPER, ZIMMERING and KRIVSHENKO 1955; FORBES 1958). Primary nondisjunction is also increased by the presence of an extra free chromosome IV (SANDLER and NOVITSKI 1956; GRELL 1959a). On the other hand, the frequency of secondary nondisjunction of the X chromosomes is decreased in the presence of heterozygous autosomal inversions (STURTEVANT 1944; OKSALA 1958; ZIMMERING 1958), or in the presence of an extra free chromosome IV (GRELL 1959a).

There is now strong evidence that these changes in frequency of nondisjunction are brought about by non-homologous pairing between the X and autosomes, and the Y and autosomes, respectively. COOPER, ZIMMERING and KRIVSHENKO pointed out that non-homologous pairing between the sex chromosomes and autosomes should also lead to autosomal nondisjunction, as would pairing between chromosomes II and III, and that autosomal nondisjunction would result in lethal aneuploid eggs. They observed large increases in egg mortality when the X chromosomes and an autosome were both heterozygous for inversions, or when the X chromosomes were structurally normal but both autosomes were heterozygous for inversions. Other causes of egg mortality were not sufficient to account for these increases. They reported the recovery of autosomally exceptional eggs, thus confirming the existence of autosomal nondisjunction in females (this was accomplished by using a special 2:3 translocation in the male which frequently gave nullo II and diplo II sperm as a result of nondisjunction). Their results, then, suggest that both major autosomes are capable of non-homologous pairing with each other as well as with the X chromosomes.

Similarly, segregation patterns in triploid females can be explained in terms of non-homologous pairing among the autosomes, and between the autosomes and sex chromosomes (SANDLER and NOVITSKI 1956). Further evidence for such interactions is given by FROST (1960).

The strongest genetic evidence for non-homologous pairing comes from data showing regular preferential segregations involving the following chromosome pairs: Y-II (OKSALA 1958), Y-IV (GRELL 1957, 1959b), X-II (FORBES 1960), Dup(X)-IV (GRELL and GRELL 1960), and X-IV (GRELL 1959a). In each of these experiments specific autosomes were made to behave as univalents with respect to their homologues by the use of translocations, or translocations and inversions. Changes in frequency of X chromosome nondisjunction were accompanied by the predicted segregation. When primary nondisjunction of the X chromosomes was increased (FORBES 1960; GRELL 1959a), the autosome was almost always found at the nullo X pole. When the frequency of secondary nondisjunction was decreased (OKSALA 1958) the autosome was almost always found at the pole opposite the Y chromosome among the eggs in which regular disjunction of X chromosomes had occurred.

Thus in females each of the four chromosomes has been implicated in the occurrence of non-homologous pairing. In the experiments reported in this paper the effects of heterozygous autosomal inversions on the frequency of sex chromosomal nondisjunction and autosomal nondisjunction were tested in males. The experiments also made it possible to test whether non-homologous pairing (as

defined by the directed segregation of two non-homologous partners to opposite poles) was responsible for the occurrence of nondisjunction in males.

Culture methods

All cultures were kept at 25 ± 1°C. Tested males were up to two days old when crossed to triploid females, cultures being transferred in five day intervals. In Experiment I single triploid females were mated to several males in creamers, and up to five such transfers were made. Matings were made in bottles in Experiments II, III, and IV and only one transfer was made. In Experiments II and IV 10 females and 20 males were crossed per bottle, in Experiment III 10 females and one male were crossed per bottle.

Method of detecting sex chromosomal nondisjunction

All triploid females carried two attached-X chromosomes and one free X chromosome and will be referred to as attached-X triploid females. The attached-X chromosomes were homozygous for the genes for yellow body color *(y)* and for apricot eyes *(w^a)*. In some triploid females the free X chromosome also carried yellow and apricot, in others the free X chromosome contained multiple inversions and was marked with yellow^{31d} *(y^{31d})* and the gene for Bar eyes *(B)*. Although the multiple inversions did not always prevent crossing over between the free X chromosome and the attached-X chromosomes with respect to Bar, such crossovers were usually detected because of other mutants present in the chromosomes. Offspring were always tested if the maternal or paternal origins of their chromosomes was uncertain. The X chromosome in the male carried yellow only. The Y chromosome carried a small duplication for the tip of the X chromosome with the wild type allele of yellow (the *sc^s*·Y chromosome).

The scheme for identifying chromosomal origins among the offspring went thus: yellow *versus* wild type determined the absence or presence respectively of the *sc^s*·Y chromosome, apricot (or Bar) *versus* wild type determined maternal *versus* paternal origins of the X chromosomes.

Effect of inversions on X-Y nondisjunction

Before examining the frequencies of X-Y nondisjunction recorded in Table 1 certain procedures used in constructing the table should be mentioned. Since equal numbers of X-bearing and Y-bearing sperm were recovered in Experiment I (13,710 and 13,410 respectively) the frequencies of XY sperm and 0 sperm are simply expressed in terms of the total number of offspring produced in the experiment. To save time the offspring in Experiments II and III were scored only for the presence of exceptions and for the numbers of attached-X diploid females. Using the ratio of attached-X diploid offspring to total number of offspring observed in Experiment I (0.245 for triploid females with the normal free X chromosome, 0.256 for triploid females with the inverted free X chromosome) the total number of offspring produced in Experiments II and III could be computed.

There are four types of males with structurally heterozygous autosomes to be compared with normal males, *Cy*/+; +/+, +/+; *Sb*/+, *Cy*/+; *Sb*/+, and *Cy*/

Gla; +/+. Curly (wings) marks the presence of *In(2L+2R)Cy*, Glazed (fused eye facets) marks the presence of *In(2LR)Gla*, and Stubble (bristles) marks the presence of *In(3R)C*. In Experiments I and II the different types of males tested were all obtained from the same parental cross. Table 1 shows that there is no indication that any of the inversions have an effect on X-Y nondisjunction. DR. KENNETH COOPER (personal communication) has also investigated the influence of autosomal inversions on X-Y nondisjunction and was also unable to find an effect.

Were an interchromosomal effect present, the inversions used in chromosome II should have demonstrated it. The Curly inversions are two large inversions, one in each arm, while the Glazed inversion is another large inversion extending across the kinetochore. The third chromosome inversion associated with Stubble is a small inversion at the tip of the right arm and does not represent a good test of the effect of structural heterozygosity for chromosome III. Since X-Y pairing only involves the heterochromatic portion of the X chromosome, one might have expected the X chromosome in males to behave similarly to X chromosomes in females that are structurally heterozygous in their euchromatic portions. This was clearly not the case. In females nondisjunction of structurally heterozygous X chromosomes is increased some tenfold when the Curly inversions are present (COOPER, ZIMMERING and KRIVSHENKO 1955) and an effect of this magnitude would have been easily detectable in the experiments reported here. Lumping together 0 and X-Y sperm recovered in Experiments I and II, there is a total of 18 exceptions from non-Curly male parents (21,425 sperm tested) and 20 exceptions from Curly male parents (23,935 sperm tested). These numbers are large enough to exclude all but a two, or at most threefold difference as possibilities.

Table 1 does show an over-all difference in the number of X-Y sperm *versus* 0 sperm recovered that is at the borderline of significance ($0.05 > p > 0.01$). The greater number of 0 sperm recovered corresponds to observations of GERSHENSON

TABLE 1

The effect of autosomal inversions on frequencies of X-Y nondisjunction. Crosses of attached-X triploid females with test males

Type of inversion in male	Experiment	$\hat{X}X$ 2N* females recovered	Number of sperm tested	Number of XY sperm	Number of 0 sperm	Total Exceptions	Percent Exceptions
+/+;+/+	I	..	6,714	3	3	6	.089
	II	2,180	8,510	2	6	8	.094
Cy/+;+/+	I	....	7,604	2	5	7	.092
	II	2,486	9,730	2	7	9	.093
+/+;*Sb*/+	I	....	6,201	2	2	4	.065
Cy/+;*Sb*/+	I	....	6,601	2	2	4	.061
Cy/*Gla*;+/+	III	2,541	10,408	1	5	6	.058
		Total	55,768	14	30	44	.079

* In Experiments II and III the number of attached-X female offspring was used to estimate the total number of offspring produced. The number of attached-X females produced in Experiment I is not given since all offspring were counted.

(1933) and SANDLER and BRAVER (1954) in experiments on X-Y nondisjunction in males. These authors utilized structurally altered X chromosomes giving high frequencies of primary nondisjunction and attributed the higher frequency of 0 sperm types to meiotic loss of unpaired chromosomes.

The method for following the inheritance of the Y chromosome had some flaws. Certain crossovers between the X and Y chromosomes mimicked nondisjunction, since they resulted in X-Y^{arm} attachments carrying the y^+ duplication and a free Y fragment lacking the duplication. Of 14 presumed cases of nondisjunction that could be tested, three turned out to represent X-Y crossovers. Consequently, the raw frequencies of X-Y nondisjunction shown in Table 1 should be reduced by something like 20 percent. This would bring the total frequency of X-Y exceptions down from 0.079 percent to 0.063 percent.

There was no significant difference between the number of X-Y exceptions collected during the 1–5 day culture period compared to the 6–10 day culture period (10 and 13 respectively for Experiments II and III). Approximately equal numbers of regular offspring were produced in both age periods.

Method of detecting autosomal nondisjunction

There were three methods used to detect autosomal nondisjunction in males. The first involved marking the chromosome sets of the males and triploid females with different sets of recessive genes. The genes used for this purpose were cinnabar *(cn)* and hairy *(h)* for chromosomes II and III, respectively, in the triploid females, and speck *(sp)* and ebony-sooty *(e^s)* for chromosomes II and III, respectively, in the male. The mutant characters associated with these genes are bright red eyes *(cn)*, extra hairs on the wings and scutellum *(h)*, a dark spot in the axil of the wing *(sp)*, and dark body color *(e^s)*. All regular offspring would be wild type with respect to these characters but exceptional diploid offspring showing cinnabar, for example, would have received both chromosomes II from the triploid parent. If possible such exceptions were always tested for the absence of the corresponding chromosomal recessive from the other parent, in this case for the absence of speck.

A second method used for chromosome II was to make that chromosome in the male homozygous for the dominant gene for lobed eyes *(L)*. All regular offspring would have lobed eyes but any offspring resulting from nullo II sperm would lack Lobe. Since Lobe is not always penetrant only progeny tested exceptions were included in the results. Diplo II sperm could not be detected by Lobe alone since the phenotypes of individuals homozygous or heterozygous for Lobe overlap badly.

The third method involved marking each chromosome in the male with a different dominant. For example, in Experiment III listed in Table 2 the chromosomes II of the male carry Curly and Glazed respectively. Offspring resulting from nullo II sperm would lack both dominants, those resulting from diplo II sperm would show both dominants.

Different combinations of these three methods were used, the particular markers present in each experiment being listed in Table 2. With one exception

TABLE 2

Frequencies of autosomal nondisjunction in males. Crosses of attached-X triploid females with test males

Exp.	Homozygous markers: Male II	Male III	Female II	Female III	Type of inversion in male	$\hat{XX}$ 2N females recovered	Type of sperm recovered: Diplo II	Nullo II	Diplo III	Nullo III	Percent nullo II
I	*L sp*	e^s	+	*h*	+	1,326	.	0	.	1	..
	L sp	e^s	+	*h*	*Sb*	1,236	.	0	.	0	..
	L sp	e^s	+	*h*	*Cy*	1,511	.	3	.	0	.24
	L sp	e^s	+	*h*	*Cy;Sb*	1,368	.	4	.	0	
					Total	5,441	.	.	.	.	.13
II	*L sp*	e^s	*cn*	+	+	4,887	.	7	.	.	..
	L sp	e^s	*cn*	+	*Cy*	4,446	(1)	6	.	.	..
					Total	9,333	.	.	.	.	.14
III	+	e^s	+	*h*	*Cy/Gla*	5,023	.	5	.	0	.10
Total (Exp. I, II, and III)						19,797	.	25	.	.	.13
IV†	+	+	+	*h*	*Cy/Pm;D/Sb*	2,219	.	7*	6	17	.32
									Nullo-III		.77

* Only X-bearing nullo II sperm could be detected. See text for explanation.
† In Experiment IV the sex chromosomes of the male were not marked.

to be mentioned, the unbalanced eggs recovered from attached-X triploid females came from the following segregation: II,II;III – II;III,III. Thus nullo II or nullo III sperm respectively were recovered as diploid offspring and could be detected by any of the methods listed above. Diplo III or diplo II sperm respectively were recovered as offspring with triploid autosomes and consequently could only be detected by the third method.

Effect of inversions on autosomal nondisjunction

In Table 2 numbers of attached-X diploid females produced by the experimental crosses are again used as a measure of the size of the experiment. The numbers do not correspond to the numbers listed for the same type of cross in Table 1 since not all crosses gave simultaneous information on X-Y and autosomal nondisjunction. In some cases only X-Y nondisjunction could be detected, and in some other cases the offspring were initially scored only for autosomal exceptions.

In the first experiment nullo II or nullo III sperm could be detected. Since all seven nullo II sperm recovered came from male parents carrying the Curly inversions, and none came from the non-Curly male parents, a second experiment was run to see if the distribution was a chance result. In this second experiment only nullo II sperm could be detected. Table 2 shows that the possible inversion effect disappeared, and taking the two experiments together there is no statistically significant difference between Curly and not Curly males.

The single diplo II sperm recovered in Experiment II is enclosed in parentheses since the frequency of diplo II sperm in that experiment is not to be compared with the frequency of the same type in Experiment III. This particular diplo II sperm was recovered because it fertilized an X;0;III egg, thus producing a diploid offspring homozygous for speck. It was possible to test this exception for the absence of cinnabar also.

In Experiment III, diplo II as well as nullo II or nullo III sperm could be detected. The recovery of diplo II sperm shows that nondisjunction rather than meiotic loss is occurring. The frequencies of nondisjunction observed here are similar to those in the previous experiments and help to confirm the lack of an inversion effect.

The fourth experiment was run to test for the occurrence of diplo III sperm as well as to test the effects of structural heterozygosity in both chromosomes II and III. Chromosomes II and III in the male were marked with *Cy/Pm;D/Sb* respectively. Plum is a dominant gene producing mottled dark colored eyes, and Dichaete is a dominant gene producing spread wings and a reduced number of bristles. The inversions carried on the Curly and Stubble chromosomes have already been described. The Plum *(Pm)* chromosome carried an inversion that included the kinetochore, *In(2LR)Pm*, and the Dichaete *(D)* chromosome carried multiple inversions covering the proximal portion of the left arm as well as most of the right arm, *In(3LR)DcxF*. Three of the four exceptional classes of sperm could be detected. Diplo II sperm could not be detected since such sperm are recovered in offspring with triploid autosomes where Plum is no longer dominant. Table 2 shows a marked increase in frequency of nullo III sperm. In previous experiments (I and III) about five times the size of Experiment IV only one nullo III sperm had been collected while in Experiment IV 17 were collected. There is also a marked increase in frequency of nullo II sperm. Only nullo II sperm carrying an X chromosome could be detected since Y carrying sperm resulted in apricot eyed offspring in which Plum could not be classified. If the proportion of nullo II sperm observed in Experiment IV is compared with the proportion of X-bearing nullo II sperm observed in the previous three experiments (11 exceptions carried X chromosomes), the former is five to six times as large as the latter.

There was no significant difference between the number of autosomal exceptions collected during the 1–5 day culture period compared to the 6–10 day culture period (31 and 23 respectively for Experiments II, III and IV). Approximately equal numbers of regular offspring were produced in both age periods.

The high frequencies of nondisjunction in Experiment IV show that interchromosomal effects may exist in males, but there are also other possibilities. Since the males used in Experiment IV came from separate stocks, genotypic differences may be exerting generalized effects on nondisjunction frequency unrelated to the structural changes in the chromosomes. In particular the Plum and Dichaete chromosomes need to be tested separately. It would be of interest to know whether the frequency of sex chromosome nondisjunction in Experiment

IV was also increased. This point could not be checked since the sex chromosomes in the male were not marked.

In Experiment IV there was evidence that exceptional offspring occurred in clusters. The 17 exceptions resulting from nullo III sperm were distributed among the 20 different cultures as follows: four cultures produced one exception, one culture produced two exceptions, one culture produced three exceptions, and two cultures produced four exceptions. In both of the last two cultures X-bearing as well as Y-bearing nullo III sperm were recovered. The occurrence of two cultures in the last category is unexpected: on the basis of a Poisson distribution, only 0.22 cultures would have been expected to have more than three exceptions. Furthermore one of these two cultures also produced two exceptions resulting from diplo III sperm, and the other culture produced two exceptions resulting from nullo II sperm. Lumping together all types of exceptions resulting from autosomal nondisjunction in the male, six cultures produced one exception, three cultures produced two exceptions, two cultures produced three exceptions, none produced four or five exceptions, and the two already mentioned produced six exceptions. Only 0.08 cultures would have been expected to produce more than five exceptions on the basis of a Poisson distribution. There was no evidence for clusters in Experiments I, II, or III with respect to autosomal or sex chromosomal nondisjunction.

The author examined the original data for STERN's 1934 paper in respect to clusters of exceptions. The 20 exceptions recorded by STERN were among 911 male offspring gathered from 246 separate triploid cultures (one female with 3–4 males). There were three clusters of two exceptions and one cluster of three exceptions. These came from cultures with a total of 5, 6, 13, and 8 males respectively, and the clusters of exceptions represented the recovery of nullo III, nullo II, diplo II, and diplo II sperm, respectively. About one cluster of two and less than 0.11 clusters of three would have been expected on the basis of a Poisson distribution.

In crosses involving triploid females a large proportion of the zygotes formed are lost and clusters of exceptional sperm would have to be fairly large in order to be detected. There are several possible explanations. If a testis showed variegation with respect to factors influencing meiotic pairing, those sectors in which normal meiotic pairing did not take place would give rise to heterogeneous clusters of exceptional sperm. Another possibility is that high frequencies of nondisjunction were characteristic for particular male parents only. Somatic nondisjunction of autosomes in spermatogonia could also lead to clusters of exceptional sperm, but a deficiency, and perhaps a duplication for an autosome, is a presumably lethal condition for spermatogonial cells.

Relative frequencies of different types of exceptional sperm

The low frequency of nullo III sperm recovered in Experiments I and III (Table 2) probably reflects a low frequency of nondisjunction for chromosome III compared to chromosome II. As pointed out earlier, exceptional sperm were recovered by fertilizing II;III,III or II,II;III eggs. Since these two egg types are the result of the same segregation they should occur with equal frequency.

Attached-X and free X chromosomes might be expected to occur with equal frequency in both egg types. Good evidence for both these points can be found in PONTECORVO's (1943) data, and the frequencies with which these egg types have been recovered in the present experiments also agree with these assumptions. This means then, that equal frequencies of nullo II and nullo III sperm would result in equal frequencies of recovered exceptions. In experiments I and III however, only one nullo III sperm was recovered compared to 12 nullo II sperm. In Experiment IV the frequencies of nullo II and nullo III sperm were equal. Table 2 shows that seven nullo II sperm were recovered but it will be recalled that only X-bearing sperm could be detected. Of the 17 nullo III sperm recovered, seven carried an X chromosome.

Another factor affecting the relative frequency of exceptions shown in Table 2 is the sex chromosome constitution of the sperm. Among diploid offspring of the experimental crosses, X-bearing sperm would be recovered half as frequently as Y-bearing sperm, since X-bearing sperm fertilizing eggs with attached-X chromosomes would be lost. Diploid metafemales (3X2A) are practically inviable. Consequently X-bearing nullo II or nullo III sperm would be recovered half as frequently as those carrying a Y chromosome. This expectation is compatible with the observation of 11 X-bearing sperm out of the 25 nullo II sperm in Experiments I, II, and III, and of seven X-bearing sperm out of the 18 cases of nullo III sperm.

Assuming that X-bearing and Y-bearing autosomally exceptional sperm are equally frequent, the above argument suggests that 75 percent of all nullo II or nullo III sperm fertilizing the proper type of autosomally unbalanced egg are actually recovered. A similar estimate for diplo III sperm in Experiment IV is 44 percent. The latter assumes 100 percent viability of triploid females, inviability of metamales, and 37 percent viability of intersexes, this last percentage being the average viability of intersexes in that experiment as determined by comparisons to the number of diploid offspring recovered from eggs produced by the same types of segregation. Although nondisjunction should produce equal numbers of nullo III and diplo III sperm, the above considerations show that the former would be recovered 1.7 times as frequently as the latter, an expectation compatible with the frequencies shown in Table 2. Records were not kept of intersex viability in Experiment III but expectations of relative recovery of diplo II sperm would be roughly similar.

Actual frequencies of nondisjunction

To know what the actual frequencies of unbalanced sperm are, one must know the frequency of the complementary type of unbalanced egg. Estimates of the latter can be made from egg counts and are discussed in detail by FROST (1960). The results can be summarized by saying that about 70 percent of the eggs formed by triploid females are unbalanced autosomally, the number of attached-X diploid female offspring being 3.5 percent of the total number of eggs laid. Making a simplified assumption that all unbalanced eggs produced by attached-X triploid females come from the II,II;III – II;III,III segregation, and that therefore 75 percent of all nullo II sperm fertilizing the first egg type are recovered, then the

frequency of nullo II sperm in Experiments I, II, and III (Table 2) is 0.017 percent. Since diplo II sperm are equally frequent the total frequency of nondisjunction for chromosome II would be 0.034 percent. These numbers represent firm minimum estimates of nondisjunction frequency since they are based on the maximum number of appropriate egg types that the triploid females could produce (see FROST 1960). Although attached-X triploid females produce other types of unbalanced eggs than the ones on which these estimates are based, their inclusion into the calculations would have only increased the third decimal figure. These estimates of chromosome II nondisjunction frequency are similar to frequencies of X-Y nondisjunction shown in Table 1. The frequencies of nondisjunction for either chromosome II or III in Experiment IV would be 5–6 times greater, i.e. 0.17–0.2 percent.

The question of non-homologous pairing

It is possible to test whether nondisjunction of a particular chromosome pair occurs independently of other chromosomes or whether nondisjunction of one pair is frequently related to nondisjunction of another pair. The latter situation could arise if different chromosome pairs responded in a similar manner to some common environmental change or, as discussed earlier, if non-homologous pairing occurred. Were non-homologous pairing responsible for nondisjunction, preferential segregations should have been observed. Let us suppose that X-Y nondisjunction was being caused by non-homologous pairing between the sex chromosomes and chromosome II. If all four chromosomes were involved in non-homologous associations all X-Y sperm should also be nullo II, and all 0 sperm should also be diplo II. If only two chromosomes were involved in non-homologous associations, and the remaining two segregated randomly, then only one half of the sex chromosomal exceptions should also be autosomal exceptions. Before turning to the results we need to ask what the relative chances are of recovery of X,Y;II;III sperm *versus* X,Y;0;III sperm, or of 0;II;III sperm *versus* 0;II,II;III sperm.

The sperm types listed above that are nondisjunctional for both the sex chromosomes and chromosomes II probably have as good a chance to be recovered as the types nondisjunctional for the sex chromosomes only. The total number of offspring recovered from attached-X triploid females represent about 14 percent of the eggs laid. Thus in terms of the number of eggs fertilized, sperm carrying a normal autosome complement have a 14 percent chance of recovery. Of the 70 percent of the eggs that are unbalanced autosomally, half are of the type complementary to nullo II sperm, and half of these in turn carry the free X chromosome which forms a viable sex type with X-Y bearing sperm. Consequently, if all eggs formed by attached-X triploid females were fertilized by X,Y;0;III sperm, 17 percent of the zygotes would be viable, while if all eggs were fertilized by X,Y;II;III sperm, 14 percent of the zygotes would be viable. If intersexes were always viable, 0;II,II;III sperm could also be recovered in 17 percent of the eggs.

In the experiments shown in Table 1 the males were homozygous for speck on chromosome II (except for Experiment III) and ebony-sooty on chromosome III. Of the 14 X-Y exceptions recorded in Table 1, progeny tests showed that one did

not carry speck, and therefore had come from an egg fertilized by a nullo II sperm (this exception came from a male parent with normal chromosomes). In only one other case could this possibility not be excluded. In only two cases could the possibility of nullo III sperm origin not be excluded. Thus the number of X-Y exceptions that were also autosomal exceptions is less than expected on the non-homologous pairing hypothesis. The frequency of 0;II;III sperm *versus* 0;II,II;III sperm could not be determined. Diplo II sperm of the latter type are recovered only in intersexes whose autosomal constitution can not be checked. Nine of the 30 nullo-XY exceptions recorded in Table 1 were intersexes.

The problem can be attacked from the other end also. If nondisjunction of chromosome II is being caused by non-homologous pairing with the X or Y chromosomes, and the remaining unpaired sex chromosome and autosome segregated randomly, then half of the nullo II sperm produced should also be X-Y in constitution. However, by analogy to the role of the Y chromosome in producing secondary nondisjunction in females (COOPER 1948), another possibility here is that since only one arm of the Y chromosome need be involved in pairing with the X chromosome, the other arm could be free to participate in non-homologous pairing. This situation would lead to Y-bearing nullo II sperm. Among the 25 nullo II sperm recovered in Experiments I, II, and III in Table 2, ten carried an X chromosome, 13 carried a Y chromosome, one was X-Y (from a male parent with normal chromosomes) and one was nullo X-Y (from a *Cy* male parent). These latter two were detected in experiments scored first for autosomal exceptions only. Even considering the single X-Y sperm in relation to the ten X-bearing sperm the proportion is below expectation. The single nullo X-Y nullo II sperm is very difficult to explain on the non-homologous pairing hypothesis.

Non-homologous pairing between chromosomes II and III should lead part of the time to nullo II diplo III sperm and *vice versa*. Such sperm were not recovered. It is not known, however, if triploid females produce the appropriate type of egg with which such sperm could be recovered if they did occur.

The recovery of three sperm nondisjunctional for more than one chromosome, two X,Y;0;III and one 0;0;III, is more frequent than expected on chance association. The frequency of the former type, however, is lower than expected if non-homologous pairing had been the major cause of nondisjunction.

DISCUSSION

The experiments reported here confirm STERN's (1934) results with respect to the four types of autosomally exceptional sperm he recovered and with respect to the fact that triploid females do produce nullo II eggs. Only one such egg was detected in these experiments, indicating a much lower frequency for these egg types than found by STERN. This difference, as well as the egg types themselves, is discussed further in a separate paper (FROST 1960). The apparent high frequency of nondisjunction in males observed by STERN remains to be explained.

The males tested by STERN carried three of the same inversions used here in Experiment IV, i.e., the ones associated with Curly, Plum, and Stubble. The

remaining chromosome III was marked with Hairless (many bristles absent) and did not carry an inversion. Since STERN's purpose in running the experiment was to test for translocations occurring in triploid females, he collected only male offspring of the experimental cross. Among 911 males collected from triploid females with three free X chromosomes, there were 20 exceptions representing the recovery of six nullo II, two nullo III, seven diplo II, and five diplo III sperm respectively. One reason already noted for the apparent high frequency of nondisjunction was that regular offspring recovered from triploid females are actually a small percentage of the total number of unbalanced eggs sampled. This effect is exaggerated when scoring exceptions only among male offspring of free X triploid females. BRIDGES (in BRIDGES and ANDERSON 1925) found that 1X1A-2X2A segregations were relatively rare compared to 2X1A-1X2A segregations but BEADLE (1934, 1935) demonstrated that in attached-X triploid females this type of preferential segregation is greatly diminished. It can be calculated from the data of these investigators that males form 24 percent of the diploid progeny of attached-X triploid females (BEADLE's 1935 data are used since the presence of Y chromosomes complicated his 1934 results) but only 11 percent of the diploid progeny of free X triploid females. In Experiment IV of the present paper males formed 30 percent of the diploid progeny. If both types of triploid females form about the same proportion of autosomally unbalanced eggs, one would have to collect at least twice as many males from attached-X triploid females in order to sample an equal number of unbalanced eggs.

Considering exceptions resulting from nullo III or diplo III sperm, STERN found seven out of 911 males, while in Experiment IV of the present paper five out of 1717 males collected were such exceptions. After correcting for the effect just described the frequencies found in the two experiments are fairly close. One further point should be mentioned which tends to counteract the above correction. In attached-X triploids only two major kinds of autosomally unbalanced eggs need be formed, II;III,III and II,II;III. In STERN's experiment 0;III and II;0 eggs are required also, and since each comes from a different segregation type, the pool of unbalanced eggs has to be divided six ways. For a given pool size, then, nullo III and diplo III sperm together would be recovered with a relative frequency of 1/6+1/6 or 1/3, while nullo III sperm in crosses with attached-X triploids would be recovered with a relative frequency of 1/2. Whatever the exact proportions of these egg types may be, the frequencies of autosomal nondisjunction obtained by STERN do not appear to be unreasonably high.

To the cases of autosomal nondisjunction in males reported here, and by STERN, can be added those found by PONTECORVO (1940). Two nullo II sperm were recovered in an experiment where about 1375 attached-X diploid female offspring were produced, and where either nullo II or nullo III sperm could have been detected. The female parents were attached-X triploids, many carrying a Y chromosome, and the male parents carried two of the inversions used here in Experiment IV, i.e., the ones associated with Curly and Dichaete.

The present studies have not provided any positive evidence for the existence of interchromosomal effects of inversions in males. The presence of heterozygous

inversions in chromosome II did not influence the frequency of nondisjunction of either the sex chromosomes or autosomes, and in Experiment IV, where both autosomes were heterozygous for inversions, the increase in frequency of autosomal nondisjunction could have been due to genotypic effects unrelated to the structural changes in the chromosomes. Genotypic effects on frequencies of nondisjunction are well known (GOWEN 1928; STURTEVANT 1929). Even assuming that the structural heterozygosity in both autosomes was responsible for the marked increase in autosomal nondisjunction obtained in Experiment IV, the interchromosomal effect thus observed was still relatively weak. According to the estimates made previously, the total frequency of sperm nondisjunctional for either chromosome II or III would be only 0.4 percent (although it should be remembered that not all types of nondisjunctional sperm, i.e., 0;III,III or II,II;0, might have been detected). Evidence from comparable situations in females indicates much higher frequencies of nondisjunction. Earlier, it was mentioned that COOPER, ZIMMERING and KRIVSHENKO found a large increase in egg mortality when females carried normal X chromosomes but were heterozygous for inversions in both autosomes. This increase, which was about 15 percent, they attributed in large part to autosomal nondisjunction. REDFIELD (1957) also found increases in egg mortality of ten percent when both autosomes were heterozygous for inversions, although she was inclined to interpret much of the increase as being due to increased crossing over within the inversions. REDFIELD had also shown that the presence of autosomal inversions in males crossed to normal females had no effect on the frequency of egg mortality. Differences of one or two percent would not have been detectable by that method, but the present experiments show that even the highest frequencies of autosomal nondisjunction would have resulted in less than one percent egg mortality. Thus even if a minor part of the increase in egg mortality observed when females carried the autosomal inversions is due to autosomal nondisjunction, autosomal heterozygosity would have a much greater effect on nondisjunction frequency in females than it does in males.

OKSALA's (1958) interpretation of interchromosomal effects in diploid females, and SANDLER and NOVITSKI's (1956) interpretation of interchromosomal effects in triploid females, are both based on the idea that pairing in heterochromatic regions is nonspecific. The fact that structural heterozygosity in chromosome II had no influence on the frequency of X-Y nondisjunction may be due to fairly specific pairing in the heterochromatic kinetochore regions in males. For the X and Y chromosomes there is both cytological and genetic evidence for the existence of several specific pairing sites (COOPER 1951; LINDSLEY and SANDLER 1958). However, the present experiments may not be a good test of the possibility that non-homologous associations could be formed in males; the reason being that in contrast to females, euchromatic pairing in males may offer little competition to heterochromatic pairing in the kinetochore regions (pairing in this region presumably being necessary for normal disjunction). Heterozygous inversions in females may delay pairing between homologous kinetochore regions, thus increasing opportunities for non-homologous associations to form, or, as in OKSALA's

model, the inversion configuration may actually bring the kinetochore regions into closer proximity with non-homologues.

Pairing between the X and Y chromosomes in males is subject to no competition from pairing sites in the euchromatic portions of the X chromosomes, and this may be sufficient reason to account for the absence of the interchromosomal effect on X-Y nondisjunction. There are also several indications that some chromosome regions in females showing strong pairing affinities have greatly weakened pairing forces in males, and that as a consequence the inversions used in the second chromosome may have been relatively ineffective in causing difficulties in pairing. Four such indications are listed below:

1. It has already been observed that structural heterozygosity in both autosomes in males results in much lower frequencies of nondisjunction than seems to be the case for females.

2. Frequencies of nondisjunction of translocated chromosome arms have been compared in male and females by DOBZHANSKY (1933) and BROWN (1940). Both found that inversions causing striking increases in nondisjunction in females had little effect in males, but most, if not all, such differences could probably be attributed to the influence of crossovers on disjunction. (The fact that the inversion effect did exist in males makes expectations of interchromosomal effects between the two major autosomes more justified.)

3. One sex difference in pairing specificities not complicated by crossing over was described by STURTEVANT (1936) who found that preferential segregations between different chromosomes IV and a duplication were much reduced in males.

4. GRELL (1957) found that the preferential segregation between a Y chromosome and chromosome IV observed in females was absent in males.

The nondisjunction that does occur in these experiments could be explained in part, at least, by the occurrence of non-homologous pairing. However, a simpler hypothesis that would account for 0;0;III as well as X,Y;0;III sperm is that nondisjunction of one chromosome pair reflects localized conditions favoring the occurrence of nondisjunction of other chromosome pairs also, and that when nondisjunction occurs involving two different pairs of homologues, they orient independently of each other. The latter explanation also fits in with the evidence that exceptional sperm can be produced in clusters.

SUMMARY

The occurrence of nullo II, diplo II, nullo III, and diplo III sperm, respectively, was demonstrated, the estimated frequency of nondisjunction for chromosome II being 0.034 percent, the frequency of nondisjunction for chromosome III being much lower. The frequency of X-Y nondisjunction was 0.063 percent.

The presence of heterozygous inversions in chromosome II had no effect on frequencies of sex chromosomal or autosomal nondisjunction. In one experiment where both chromosome II and III were heterozygous for inversions, there was a sixfold increase in frequency of nondisjunction for chromosome II, and chromo-

some III nondisjunction increased to a frequency equal that for chromosome II. The effect on sex chromosomal nondisjunction in the latter case was not determined, and whether genotypic differences rather than structural heterozygosity were influencing nondisjunction frequency is unknown. The experiment also gave evidence that autosomally exceptional sperm were being produced in clusters.

Three sperm nondisjunctional for both chromosome I and II were recovered, two were X,Y;0;III and one was 0;0;III. Their frequency was much greater than expected by chance association, but the frequency of the former type was lower than expected if non-homologous pairing had been the major cause of nondisjunction.

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SELECTION FOR BLOOD-pH IN THE HOUSE MOUSE[1]

H. G. WOLFE[2]

The Hall Laboratory of Mammalian Genetics, University of Kansas, Lawrence, Kansas[3]

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TWO-WAY selection for blood-*p*H in the house mouse was undertaken with the following objectives.

1. *Replicate the selection experiment of WEIR and CLARK (1955):* The true test of any work is its repeatability. If the results of a second investigation differ from those of the first, the finding of causes for the difference may give new insight into the problem.

2. *Determine the nature and extent of selection limits:* Limits may not have been realized in the selection by WEIR and CLARK since their experiment was carried through only three generations. Selection in the current experiment was performed for seven generations. In addition, minimal inbreeding circumvented premature fixation of undesirable gene combinations.

3. *Determine the mode of inheritance of blood-pH by means of standard genetic crosses:* The efficacy of these crosses would be limited because the lines would not be inbred. Allelism and the extent to which the current experiment replicated the first experiment might be determined by appropriate crosses between lines of the two selection experiments.

4. *Ascertain if a modified sex ratio would result from a second selection:* Differences in sex ratio had arisen as correlated responses to selection for high and low blood-*p*H (WEIR 1953).

The experiment essentially replicated the earlier selection experiment of WEIR and CLARK (1955), except for the following differences:

Selection by WEIR and CLARK	Selection by WOLFE
1. Intense inbreeding was practiced.	Minimal inbreeding was practiced to generation 6.
2. Samples consisted of venous blood from the heart.	Samples consisted of arterial blood from the heart.
3. Selection was terminated after three generations.	Lines have been selected for seven generations.

This investigation is not intended as a critique of the genetical basis of selection but does touch on current theory. Principal interest centers on the mode of inheritance of blood-*p*H and the nature of the correlated response, sex ratio.

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[2] National Science Foundation Cooperative Fellow 1959–60.

[3] Present address: Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

Most information on selection in mice comes from body weight experiments. Of the several selection experiments for body weight in mice, the best known are those by GOODALE (1938), MACARTHUR (1944), and FALCONER (1953a). Selection for lactation in mice has been investigated by BATEMAN (reported by FALCONER 1953b and 1955). Two-way selection for litter size by FALCONER (1955) was effective although response in the first two generations of selection was opposite to that expected.

Effectiveness of selection for ovarian response to a standard dose of gonadotrophic hormone in the rat has been reported by KYLE and CHAPMAN (1953). Recently, RODERICK (1959) studied the response in rats to high and low selection for cholinesterase in the cerebral cortex. SUNDER (1960) successfully selected for high and low levels of thyroid activity (I^{131} retention) in the rat. Selection in the latter was accompanied by complete sterility in the fifth generation with no living young produced.

MATERIALS AND METHODS

Foundation population and method of selection: The two selected lines, the outbred foundation stock and high and low blood-*p*H lines derived by WEIR and CLARK (1955) are designated as follows:

T — foundation stock (from Toronto) and common origin of all selected lines

TH — high blood-*p*H
TL — low blood-*p*H
} lines selected by WOLFE

PHH — high blood-*p*H
PHL — low blood-*p*H
} lines selected by WEIR and CLARK

The outbred T stock was obtained originally from MACARTHUR (1944) who established the line by crossing seven laboratory strains of mice. It has been maintained as an outbreeding colony since the time it was obtained in 1949. Selection by individual merit was practiced, beginning with the T stock. Positive assortative matings, with minimal inbreeding, were made up to generation 6. Progenies of generations 6 through 8 were products of full-sib matings. The selected lines have been maintained by means of 11 to 13 monogamous matings in each generation, and only one litter from each mating was reared and tested, with a few exceptions. The T line has been maintained continuously to serve as a control to the selected lines. F_1 crosses were made from parental lines of generation 4; F_1 and F_2 crosses and backcrosses were made from lines of generation 5. The F_1 progeny from the latter (appearing in generation 6) were not carried to weaning but were sacrificed after verification of sex at about two weeks of age. In generation 7, progenies were obtained from crosses of PHH and PHL with TH and TL lines. In addition, some PHH and PHL mice were tested for blood-

*p*H. Polygamous matings were made from T line parents of generation 7 and heritability estimates were obtained from the *p*H testing of their progeny appearing in generation 8. No selection was practiced subsequent to generation 7.

One half of the foundation population (population size 511 as of weaning) was tested for blood-*p*H, of which 73 determinations were classified as arterial. Thereafter approximately 90 percent of the individuals in each weaning population were successfully tested for either arterial or venous blood-*p*H.

Generations of selection are numbered consecutively beginning with generation 0 (foundation population). Progenies from crosses and unselected lines are assigned the generation number of the selected lines to which they are contemporaneous as a point of reference.

Measurement of blood-pH: Mice were usually 60 to 120-days old at the time their blood was tested. Samples of arterial blood were collected from the heart by means of a hypodermic needle and syringe after the method employed by WEIR (1949). Some samples of venous blood were collected but these mice were not used in selection when it could be avoided. In those few cases in which venous blood was used, corrections to selection differentials were made.

Measurements of blood-*p*H were made at room temperatures on nonanaesthetized mice, using a McInnes-Belcher micro-glass electrode. A room temperature reading to the nearest 0.5°C was taken for each sample of blood tested. No anticoagulants were added to the blood, but before the taking of each sample the syringe and needle were rinsed in a three percent solution of potassium oxalate. Details of the method, including problems encountered in the measurement of *p*H of the blood, have been described (WOLFE 1959).

The effect of temperature on blood-*p*H *in vitro* was removed statistically by linear regression. Data were pooled to obtain the best possible estimate of $\beta_{y \cdot x}$, and sample regression coefficient of *p*H on temperature was calculated to be −.010. All blood-*p*H means are adjusted to a standard temperature of 25.8°C (grand temperature mean) unless noted otherwise. No effect of age on blood-*p*H was demonstrated by regression and age differences are therefore disregarded.

Methods of sampling mice: Methods of capturing and handling mice, or the order in which they are tested if all mice of a cage are tested consecutively, may affect the *p*H of the blood. It is relevant, therefore, to describe the methods of sampling used, which varied somewhat from generation to generation.

Litters of mice are normally weaned at 30 days. The usual procedure is to house all the weaned members of one sex together in a cage until a capacity of about six mice is reached. Usually litter mates of one sex remain together or are contained in no more than two cages.

In sampling the foundation stock (generation 0), all mice of a cage were tested consecutively from cages chosen at random. In generations 1 through 3, TH, T and TL mice were housed separately and mice of a cage were sampled consecutively. Cages of a line were chosen alternately. To distribute environmental effect and provide ease in testing, mice of generations 4 and 5 were each interspersed through the cages (six mice per cage) shortly after weaning. The relationship among mice of a cage for line, age, and parentage was entirely random. Blood-*p*H

determinations were made for all the mice in a cage, then mice of another cage were tested. Mice of generation 6 were randomized in the same way as mice of generations 4 and 5 except that each mouse was segregated into an individual cage usually 24 hours and no more than one week prior to testing for *p*H. In generation 7, mice were not interspersed through the cages as were mice of the preceding three generations. Lines of mice were housed separately, but mice within lines remained together as weaned. For the estimates of heritability of blood-*p*H in a random-bred population, T mice of generation 8 were housed six to a cage soon after weaning in such a way that distribution of families and littermates was random. Mice were then drawn randomly from the "population" of cages. Each mouse was replaced in a cage after testing. This elaborate procedure was followed so that any maternal effect would be minimized and other environmental variance common to sibs would be randomly distributed throughout the population.

Response to selection

Two-way selection for high and low blood-*p*H was practiced without interruption for seven generations. Means and standard errors of the two selected lines plus those of the control line are given in Table 1. No *p*H means were obtained for the control line in generations 1 and 2. There is no detectable difference in blood-*p*H between sexes, and this confirms an earlier report by WEIR (1949) and WEIR and CLARK (1955). Data from sexes are therefore pooled in all analyses.

Response to selection was immediate. This is evident from Table 1, which shows the difference between adjusted means of the TH and TL lines in each generation of selection. The first generation of selection resulted in a divergence between means of .029 *p*H units, which is more than three times the standard error of the difference. There was no significant change in generation 2, but progress continued thereafter through generation 4. Maximum difference between the means of the TH and TL lines was .069 *p*H units and was attained in generation 4. No further progress was made in three additional generations of selection. The differences in blood-*p*H among adjusted means of generations 4 through 7 (range of .063 to .069) are not significantly different and may be considered as estimates of the same parameter. A graphic resumé of selection progress is presented in Figure 1.

Symmetry

It would appear from Figure 1 that no progress was made in the low line, were it not for the control-line means in generations 3 through 7 which fall midway between the two selected lines. If the assumption is made that little or no change in gene frequencies for blood-*p*H occurred in the foundation stock (control line) from the time of generation 0, then an alternative explanation must be offered. The obvious approach is to examine selection differentials that might differ in magnitude in the two lines as well as between generations within lines. Selection differentials and response to selection for each line are given in Table 2. It can be seen that the response as measured for each line varied greatly between genera-

TABLE 1

Selection for blood-pH

Generation	Line	N	Mean temp. (°C)	Arterial mean blood-*p*H	High minus low
0	T	73	25.2	7.485 ± .0066	. . .
1	TH	65	27.2	7.539 ± .0058	.029**
	TL	62	27.3	7.510 ± .0069	
2	TH	93	25.1	7.548 ± .0045	.023***
	TL	77	25.0	7.525 ± .0050	
3	TH	78	24.8	7.550 ± .0051	
	T	61	24.9	7.518 ± .0060	.055***
	TL	69	24.8	7.495 ± .0056	
4	TH	64	26.8	7.582 ± .0062	
	T	72	26.7	7.548 ± .0066	.069***
	TL	86	26.9	7.513 ± .0054	
5	TH	96	26.0	7.576 ± .0048	
	T	56	26.0	7.533 ± .0052	.063***
	TL	89	25.9	7.513 ± .0051	
6	TH	54	26.7	7.568 ± .0076	
	T	58	26.4	7.535 ± .0056	.063***
	TL	72	26.4	7.505 ± .0056	
7	TH	52	24.9	7.552 ± .0070	
	T	68	25.2	7.519 ± .0059	.063***
	TL	47	25.1	7.489 ± .0072	

** $P < .01$.
*** $P < .001$.

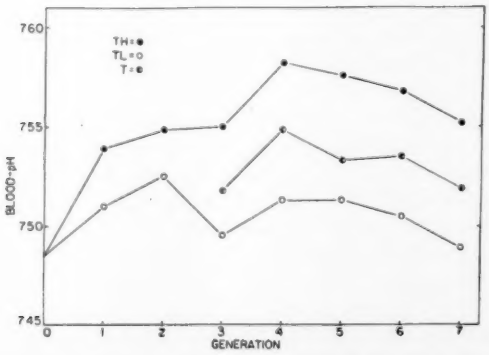

FIGURE 1.—Response to selection. A plot of adjusted blood-*p*H means *versus* generation time. The unselected T line is included as a control.

TABLE 2

Selection differentials and response to selection

Generation	Line	Selection differential† (*p*H units)		Response‡ (*p*H units)	Combined selection differential (*p*H units)	Combined response (*p*H units)	Heritability (percent)
1	TH	.071	(.068)	.054	.224	.029	12.9
	TL	.153	(.149)	—.025			
2	TH	.046	(.042)	.009	.085	—.006	— 7.0
	TL	.039	(.038)	—.015			
3	TH	.045	(.043)	.002	.097	.032	33.0
	TL	.052	(.046)	.030			
4	TH	.041	(.042)	.032	.087	.014	16.1
	TL	.046	(.046)	—.018			
Subtotals					.493	.069	14.0
5	TH	.026	(.027)	—.006	.072	—.006	...
	TL	.046	(.050)	0			
6	TH	.011	(.015)	—.008	.051	0	...
	TL	.040	(.043)	.008			
7	TH	.018	(.022)	—.016	.051	0	...
	TL	.033	(.032)	.016			

† Selection differentials are weighted proportionately to the number of tested offspring contributed by each mated pair. Expected (unweighted) differentials are shown in parentheses.
‡ Difference in blood-*p*H means between parental and offspring populations, in the predicted direction.

tions. The summed selection differentials are actually larger in the TL line, the line that gave less apparent response. Excepting generation 1, differentials through generation 4 are similar for TH and TL. The over-all increase in mean blood-*p*H for both lines in generation 1 (Figure 1) is probably due to better technique. The apparent lack of progress in the TL line may be attributed to misclassification of type of blood (arterial *versus* venous) in a few instances. Venous blood is approximately .09 *p*H units less alkaline than arterial blood, depending on strain (WOLFE 1959 and data this experiment). The use of some venous blood samples in choosing TL mice for mating would have the effect of lowering selection pressure and causing an overestimation of selection differentials in the low blood-*p*H line.

Errors in scoring of phenotype are not likely to have affected the estimation of population means in the selected lines. To avoid bias, *p*H readings were first made for a sample of blood and recorded before it was known to which strain the mouse belonged.

Several other known causes of asymmetrical response to selection should be

mentioned. Aside from an unsuitable metric, unequal initial gene frequencies and directional dominance may be sources of asymmetry (FALCONER 1953a and 1953b). If the population of alleles controlling a character is of greater frequency for one direction, response to two-way selection will not likely be symmetrical in a long term experiment. Such a situation might exist in an experiment where the character had been subject to a prior history of selection (FALCONER 1953a). Directional dominance or directional overdominance are presumed to be present if a character shows inbreeding depression in the absence of selection. These causes can be ruled out as an important source of asymmetry since response was of short duration and there was no observed reduction in fitness up to the time a selection plateau was reached in generation 4.

Since there is no way of determining the extent of the error in the selection differentials of the TL line, it seems pointless to pursue the question of symmetry further. Asymmetry of response between high and low lines is not indicated on the basis of available evidence.

Heritability

Realized heritability (FALCONER 1953a,b) is the ratio of selection response to selection differential. Due to errors in classification of type of blood unique to selection differentials of the TL line, heritability between TH and TL lines cannot be compared with any degree of certainty by this method. Assuming heritability to be the same in the two lines in the absence of evidence to the contrary, a reasonably good estimate, albeit an underestimate because of the TL line, can be obtained from the ratio of combined (TH + TL) selection response to cumulative selection differential. In this way, intergeneration variation becomes of lesser importance to selection response in that one is measuring only the absolute difference between the two lines without regard to the means of the populations from which they were derived. In the computation of selection differentials, midparental *p*H values were adjusted to a standard temperature of 25.8°C and differentials weighted according to the number of offspring contributed by each parental pair. Realized heritability for each generation of selection through generation 4 is given in Table 2. No further progress was made subsequent to generation 4, and calculations are valid only to this point. Although continual selection pressure was applied as indicated by the differentials in generations 5, 6, and 7, effective upper and lower limits to selection had been reached. There was no diminishing response through generation 4 and response appears to be linear. A heritability estimate of 14.0 percent is obtained from the ratio of summed response to summed selection differential for generations 1 through 4.

To obtain a better estimate of realized heritability, linear regression coefficients were calculated for combined cumulative selection response (CCSR) and combined cumulative selection differential (CCSD) as shown in Figure 2. The ratio of regression coefficients of CCSR to CCSD gave a realized heritability of 16.8 percent.

Heritabilities were also calculated from intraclass correlations in the progeny of polygamous matings of the T line of generation 7 (seven matings, two females

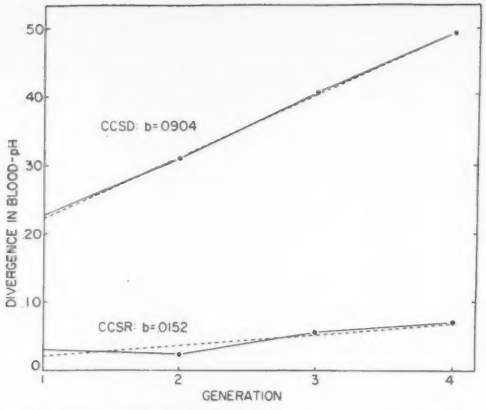

FIGURE 2.—Combined cumulative selection differential (weighted) and divergence or combined cumulative selection response per generation. Realized heritability is calculated as the ratio b_{CCSR}/b_{CCSD}.

to one male, for a total of 101 tested progeny). This method of calculation is based on the assumption that the genetic variance between full sibs is one half that of the whole population. Since there is some inbreeding in the T line, estimates will be biased in the minus direction. Heritability estimates were 29.0 percent based on variance between dams compared to zero percent based on variance between sires. For dam and sire variance combined, heritability was 14.5 percent. The discrepancy between estimates, though not statistically significant, gives some indication that maternal or nonadditive genetic effects are present.

As a means of checking for maternal effects, between-litter variances were compared to within-litter variances in the inbred PHH and PHL strains tested in generation 7. If maternal effects are present, then sibs should show a smaller variance than nonsibs. Between-litter variances were found to be equal to the within-litter variances within sampling error, even though litters remained together after weaning and shared a common cage environment to the time of testing. The latter would serve to confound and reinforce any maternal effect present.

WEIR (1949) found that roughly 40 percent of the variance was due to genetic differences between strains in his analysis of venous blood-*p*H in five highly inbred strains of mice. Error variance was about 50 percent whereas ten percent of the variance was attributable to day of test. The five inbred strains have separate origins and were derived independently of the level of blood-*p*H. They represent in theory five nearly homozygous strains chosen at random from a hypothetical population of strains. The genetic variance of 40 percent between strains is therefore equal to heritability in the broad sense.

The heritability estimates derived from phenotypic correlations among sibs

agree well with realized heritability, but the estimate from comparison of inbred strains is greater than realized heritability. The latter may be due in part to inbreeding in the T population. Barring inbreeding, the extent to which realized heritability under a completely additive scheme falls short of estimates based on other methods might be indicative of (1) error in selection differentials of the TL line from misclassification of type of blood, (2) negative correlations between multiple targets of selection, or (3) the effect of natural selection against extreme or unbalanced genotypes.

Results from crosses

F_1, F_2, and backcrosses: In the fourth generation of selection, mice of the TH and TL lines were crossed to produce F_1. Since the lines were not homozygous for the genes affecting high or low blood-*p*H or on a uniform genetic background, the F_1 individuals are not F_1 in the strict sense. It was hoped that some measure of directional dominance (net phenotypic dominance) or overdominance could be ascertained. Following this, F_1 individuals were mated to produce F_2 (contemporary to generation 6). At the same time, F_1 individuals were backcrossed to parental mice. Results of the crosses are summarized in Table 3.

Reciprocal matings were made for each of the crosses; no significant differences in blood-*p*H among progenies from reciprocal matings were found. Consequently, reciprocals are pooled and no distinction between them is made in Table 3. One may either compare progeny means from crosses with the midparental *p*H or compare them with *p*H values of parental lines tested in the same generation. With respect to the former, it is necessary to adjust blood-*p*H means to a common temperature mean (25.8°C) because of intergeneration differences in temperature; this has been done in Table 3. In some ways, comparison with "contemporary midparent" is to be preferred since environmental variance is distributed at random among contemporary mice. If such comparisons are made, one must make the assumption that little or no net genetic change occurred in the selected lines from one generation to the next. One must additionally make the dubious assumption that parents sampled from parental populations represented a true genetic cross section.

TABLE 3

F_1, F_2, and backcrosses

Cross	N	Mean temp. (°C)	Arterial mean blood-*p*H	Midparent	"Contemporary midparent"†
P_H‡	9	26.8	7.610 ± .0321	.....	....
B_H	28	26.4	7.547 ± .0086	7.576 ± .0090	7.552
F_1	78	26.0	7.544 ± .0057	7.534 ± .0041	7.544
F_2	65	26.4	7.539 ± .0050	7.542 ± .0100	7.536
B_L	32	26.5	7.535 ± .0076	7.527 ± .0092	7.520
P_L‡	9	26.7	7.468 ± .0276	.....	....

† "Contemporary midparent" blood-*p*H of F_1 and F_2 was calculated from contemporary TH and TL lines as $\bar{x}_{TH}+\bar{x}_{TL}/2$; "contemporary midparent" blood-*p*H of B_H and B_L was calculated as $\bar{x}_{TH}+x_T/2$ and $\bar{x}_T+\bar{x}_{TL}/2$ respectively.

‡ Polygamous matings for F_1 were made reciprocally in the ratio of 2♀♀:1♂ for a total of 12 matings.

Mean blood-*p*H for F_1 was greater than the midparent or the blood-*p*H mean (7.533) of the contemporary T line of generation 5, but equal to the "contemporary midparent." Differences were not statistically significant. Segregating F_2 progeny had a blood-*p*H mean somewhat lower than F_1 and midparent but were not significantly different from them. Blood-*p*H means of the contemporary T line (7.535) of generation 6 and "contemporary midparent" were similar to F_2. Backcross progenies (B_H and B_L) had blood-*p*H means which were equal to the midparent, within the limits of sampling error.

There is no indication of either directional dominance or overdominance among loci determining blood-*p*H from the results of the crosses above. WEIR (1949) found that blood-*p*H means of F_1 from crosses between three inbred strains of mice not selected for blood-*p*H were greater in two of the three possible crosses. In the F_1 of crosses between PHH and PHL, blood-*p*H means were significantly lower than the low-*p*H parent (WEIR and CLARK 1955).

In Figure 3, normal curves are superimposed over histograms of TH and TL lines of generation 4, and for F_1 and F_2 progenies appearing in generations 5 and 6 respectively. The ungrouped frequency distributions of the TH and TL lines are each slightly skewed, the former to the right and the latter to the left, and the

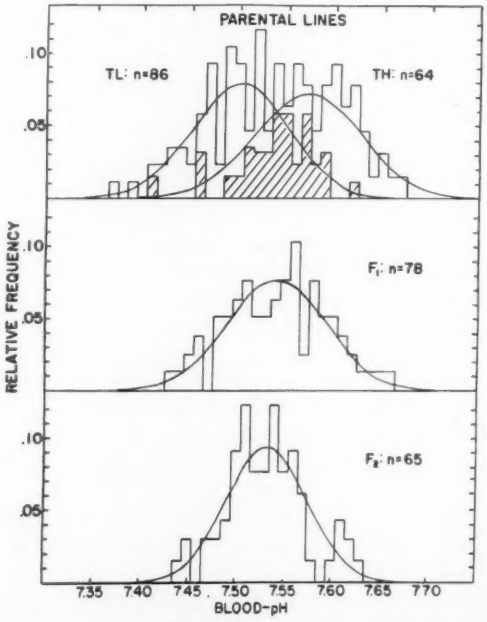

FIGURE 3.—Histograms of TH, TL, F_1 and F_2 with superimposed normal curves. Data are unadjusted for temperature. The frequency distributions are normally distributed as tested by chi-square.

two distributions merge. Range of the TH line was 7.41 to 7.67 for the 64 individuals tested; the range was 7.37 to 7.62 for 86 individuals tested in the TL line. Blood-*p*H of the F_1 was quite variable with a range of 7.43 to 7.66 but within the bounds of the TH and TL parental lines. Range of the F_2 (7.44 to 7.63) was less than the F_1 and total variance was correspondingly less. Distribution of the F_2, a population composed of 65 tested individuals, corresponds very closely to the distribution of the control (T) line (58 individuals), with reconstitution of phenotypes. F_1 had a variance equal to the TH and TL lines while segregating F_2 had a smaller variance equal to that of the T population which it simulates. Differences among variances are not significant.

From TH and TL lines of generation 6, crosses were made with PHH and PHL strains. Ranking of progeny means from high to low *p*H differed depending on whether comparisons were made on arterial or venous blood. Consideration of both arterial and venous blood-*p*H simultaneously is best accomplished by a comparison of arterio-venous (A-V) differences. Lines and strains of contemporary mice served as controls.

Arterio-venous differences in blood-pH

It was noted early in the experiment that arterio-venous (A-V) differences in blood-*p*H were of different magnitude in the selected lines. This was suspected after a significant difference was discovered between PHH and PHL strains in a concurrent experiment and gave rise to the supposition that selection for blood-*p*H on an arterial basis produces a result different from selection on a venous basis (WOLFE 1959). Selection on an arterial basis in the present experiment caused some directed change in venous blood-*p*H, but the change is less than that for arterial blood-*p*H. Thus PHH A-V < PHL A-V and TH A-V > TL A-V.

WOLFE (1959) reported that arterio-venous differences in blood-*p*H in the PHH strain was .053 *p*H units compared to .091 *p*H units for the PHL strain. The blood-*p*H data from PHH and PHL strains (tested randomly with populations of generation 7) presented in Table 4 confirm the earlier findings. Arterio-venous difference for PHH is .061 *p*H units whereas arterio-venous difference for PHL is .085 *p*H units. All of the difference between strains (.024 *p*H units) comes from a difference in venous blood-*p*H. Arterio-venous differences between strains are statistically significant.

Arterio-venous difference in blood-*p*H for data from generations 4 through 7 (generations of maximum divergence between TH and TL lines) were combined

TABLE 4

Arterio-venous differences in blood-pH in PHH and PHL

Line	N	Arterial mean	N	Venous mean	Difference
PHH	16	7.526	16	7.465	.061
PHL	17	7.527	17	7.442	.085
PHL A-V minus PHH A-V = .024*					

* $P < .05$.

as seen in Table 5. Results may be expressed as follows: TH A-V > T A-V > TL A-V. TH and TL arterio-venous differences differ significantly, and this stems from a significantly greater interstrain difference in arterial blood-*p*H over that of venous blood-*p*H. It is noteworthy that the magnitude of the arterio-venous difference in the T line is midway between that of the TH and TL lines.

Results of arterio-venous differences for crosses of TH and TL with PHH and PHL (interselection crosses) are given in Table 6. These differences bear out the general hypothesis, except that TL × PHH and TH × PHH crosses gave results opposite to those expected. However, it must be pointed out that numbers are small and confidence limits rather wide so that one must use caution in their evaluation. There does not appear to be a "reinforcement" in TH × PHL and TL × PHH crosses which one might expect if the genetic contribution of each strain in the cross served to push blood-*p*H in a given direction independently or semi-independently to that of the strain to which it was crossed, assuming there is no allelism or a limit brought about by natural selection.

TABLE 5

Arterio-venous differences in blood-pH in TH, T, and TL

Line	N	Arterial mean	N	Venous mean	Difference
TH	266	7.571	31	7.478	.093
T	254	7.534	48	7.448	.086
TL	294	7.507	59	7.430	.077

TH A-V minus T A-V = .007
T A-V minus TL A-V = .009
TH A-V minus TL A-V = .016**

** P < .01.

TABLE 6

Arterio-venous differences in blood-pH in crosses of TH and TL with PHH and PHL

Cross	N	Arterial mean	N	Venous mean	Difference
TH × PHL	29	7.516	11	7.425	.091
TL × PHL	21	7.505	21	7.427	.078
TL × PHH	21	7.512	23	7.439	.073
TH × PHH	18	7.518	16	7.464	.054

TH × PHL A-V minus TL × PHL A-V = .013
TH × PHL A-V minus TL × PHH A-V = .018
TH × PHL A-V minus TH × PHH A-V = .037***
TL × PHL A-V minus TL × PHH A-V = .005
TL × PHL A-V minus TH × PHH A-V = .024*
TH × PHH A-V minus TH × PHH A-V = .019

* P ≤ .05.
*** P < .001.

Effect of selection on phenotypic variance

WEIR and CLARK (1955) observed that progeny from unrelated parents were less variable in blood-*p*H than progeny from full sib and half sib matings. WEIR (1949) obtained contradictory results in F_1 progeny from crosses of three inbred strains of mice. S × Ba crosses gave a smaller variance in F_1 than in the parental strains; S × Z and Ba × Z crosses gave a greater variance in the F_1. These facts taken together seemed to indicate an influence of homozygosity or heterozygosity *per se* (WEIR and CLARK 1955). Greater variation in blood-*p*H in crosses involving the Z strain may be due to a heritable instability of the Z strain (WEIR 1949).

The absolute necessity for a "steady state" of *p*H of the blood indicates that the stability of the character may be maintained in part by heterozygotic balance and a complex system of gene-environment interactions. It might also be argued that the efficiency of buffer systems in the blood obviates such a need. Phenotypic variances of TH and TL lines either remained the same or increased slightly subsequent to generation 1 while they remained relatively constant in the control line. There was no decrease in phenotypic variance despite an expected reduction in between-litter variance from inbreeding increment (F) as selection advanced. Slightly larger variances in early generations that decreased rapidly to generation 2 are probably a result of a combination of better technique and better temperature control and not to a reduction in genetic variance. Data from inbred PHH and PHL mice tested in generation 7 showed smaller variances in *p*H than TH, T, TL, and crosses. The results indicate that inbred strains may be less variable for blood-*p*H than segregating or outbred populations and weaken, but do not invalidate, the case for genetic homeostasis. Phenotypic variances of F_2 and backcrosses were less than F_1 or parental variances (see Figure 3).

Data are not available on the extent to which natural selection might cause blood-*p*H in the TH and TL lines to revert to more intermediate phenotypes upon relaxed selection. If an analagous situation may be used, blood-*p*H of the PHH and PHL lines of WEIR and CLARK did not show extensive regression (WOLFE 1959). As reported by WEIR and CLARK (1955), the mean difference between high and low blood-*p*H lines was .050 *p*H units after three generations of selection. A difference of .043 *p*H units was found to persist in venous blood after 14 to 21 generations of inbreeding from the time selection ceased (WOLFE 1959). The PHH and PHL lines were maintained principally by brother-sister matings through the interim. Data from the present experiment on the same PHH and PHL lines after some 20 to 27 generations of inbreeding indicate a greater amount of regression. For PHH and PHL mice tested in generation 7, difference in venous blood-*p*H between strains was .024 *p*H units. This represents a regression of approximately 50 percent.

Correlated responses

Sex ratio: Concomitant with selection for blood-*p*H was a significant shift in sex ratio from the theoretical 0.5. As used here, sex ratio is expressed as the proportion or percentage of males. Litters were weaned at 30 days and most sex ratios shown are those at weaning. "Birth" ratios are from litters normally sexed

within 12 hours of birth, and all progeny (live and stillborn) are included in the data when it was possible to determine the sex. It has been found that sexing at birth can be done with fair accuracy. Surviving members of a litter were checked at about ten days of age and ratios corrected on those few occasions when a discrepancy between the two ratios was evident. At this time, the female sex can readily be distinguished by the presence of mammae which show through the thin coat of abdominal hair.

Figure 4 shows the deviations in sex ratio in the TH and TL lines as selection progressed. The TH line has a lower percentage of males than the TL line. This contrasts to the sex ratio of PHH and PHL strains where high blood-*p*H (PHH) is associated with a higher percentage of males than the low blood-*p*H line (PHL) (Weir 1953). Although the sex ratio in Figure 4 is variable from generation to generation, the curves of the TH and TL lines roughly parallel one another and do not intersect through generation 7. In generation 8 the sex ratio characteristics of the TH and TL lines reversed. No selection was practiced in generation 8; consequently blood-*p*H data are not available for comparison. The lines continue to be under observation, and any speculation as to causes for the apparent change would be premature at this point.

Further examination of Figure 4 reveals that the inequality of sexes appeared immediately and did not seem to increase as the two selected lines diverged in blood-*p*H. The T (control) line is included for comparison. The sex ratio of the T line is highly variable and transgresses both the TH and TL lines at different points. The general effect is to cause an equality of sexes over a period of time which approaches closely the theoretical value of 0.5.

Table 7 gives sex ratio differences between TH and TL lines in each generation of selection. Although the difference in any one generation is not statistically significant, the pooled data are significant at the .02 level of probability. The fact that the two curves parallel one another with the notable exception of gener-

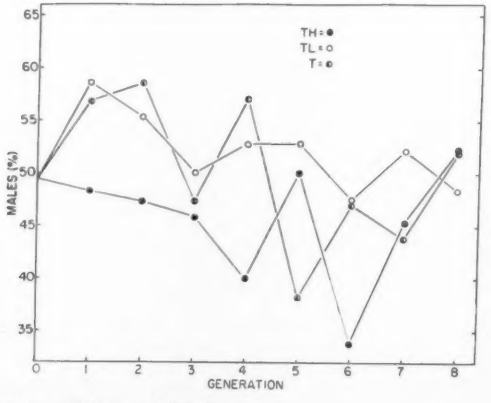

Figure 4.—Sex ratios of TH, T, and TL lines.

TABLE 7

Differences in sex ratio between TH and TL lines

Generation	Line	No. of litters	Males/ total	Males (percent)	TL minus TH (percent)	Chi-square test of difference	
	TH	11	42/87	48.3			
1	T	12	63/111	56.8	10.3	1.87	
	TL	9	51/87	58.6			
	TH	12	53/112	47.3			
2	T	6	24/41	58.5	8.0	1.38	
	TL	12	57/103	55.3			
	TH	11	39/85	45.9			
3	T	12	46/97	47.4	4.1	0.27	
	TL	11	38/76	50.0			
	TH	9	26/65	40.0			
4	T	12	44/77	57.1	13.8	2.83	
	TL	12	50/93	53.8			
	TH	12	52/104	50.0			
5	T	11	29/76	38.2	2.8	0.17	
	TL	13	56/106	52.8			
	TH	10	25/74	33.8			
6	T	11	48/102	47.0	13.8	3.41	
	TL	12	50/105	47.6			
	TH	11	35/77	45.4			
7	T	12	47/107	43.9	6.6	0.68	
	TL	11	47/90	52.2			
	TH	8	34/65	52.3			
8	T	16	66/127	52.0	—3.8	0.19	
	TL	9	33/68	48.5			
						10.80	Total
	TH	84	306/669	45.7			
1–8	T	92	367/738	49.7	6.8	6.32**	Pooled
	TL	89	382/728	52.5			
						4.48	Heterogeneity

** $P < .02$.

ation 8 indicates homogeneity. This is borne out by a small heterogeneity chi-square. The latter fact validates the hypothesis that a heritable change was effected in the lines selected for blood-*p*H.

Sex ratio data from crosses were too few to draw any conclusions but data are accumulating; a more complete treatment of sex ratios involving TH, T, and TL lines and crosses will be presented elsewhere.

Fecundity and viability: There was no observable change in fitness in the TH and TL lines through generation 5 (maximum progress was attained in generation 4). Criteria were number of sterile matings, mean litter size at birth, and survival from birth to weaning. With the advent of inbreeding (beginning with generation 6), there seems to have been a change in mean litter size at birth in TH and TL lines, especially generation 8. An unusual number of stillbirths and cannibalized litters also occurred in generation 8 in the TH and TL lines. Two possibilities exist, neither of which can be ruled as the unequivocal cause: some sort of genetic crisis occurred in the selected lines, possibly due to the effects of inbreeding, or environmental circumstances were responsible for the increased mortality of the young. The contemporary T line (outbred) did not show any reduction in fecundity or viability although some trouble was experienced in other contemporary matings of TH and TL with PHH and PHL lines.

If extreme blood-*p*H in either the high or the low line is being acted against by natural selection, then the relative contribution of the most extreme parents to the population of the next generation should be less than that of the less extreme parents. This should show up in Table 2 as a discrepancy between expected and effective selection differentials. Although expected differentials exceed effective differentials in generations of selection subsequent to generation 4, differences are well within the limits of sampling error.

DISCUSSION

Mode of inheritance: The rapidity of response coupled with early limits to selection indicates that few loci are involved in the determination of blood-*p*H. This confirms the conclusion reached by WEIR and CLARK (1955). Most progress in their experiment was confined to the first generation of selection (divergence between PHH and PHL means = .046 *p*H units) and greatest divergence was .052 *p*H units reached in the second generation. This compares to a first generation divergence between TH and TL lines of .029 *p*H units with maximum divergence in generation 4 of .069 *p*H units. Arterial blood was used in the present experiment while venous blood was used in the earlier selection experiment (see first part for an enumeration of differences between the two experiments).

The use of a scale is arbitrary, but the best scale is one on which the effects of factors (genetic and environmental) are as nearly additive as possible (WRIGHT 1952). Equality of F_1, F_2, and backcrosses to their respective midparental values indicates that the use of raw data without transformation is justified. Frequency distributions of TH, TL, F_1 and F_2 in Figure 3 show a continuum and do not differ significantly from superimposed normal curves. Variances are not proportional to the means, i.e., coefficients of variation do not change the relationship of means of one line or cross to another. Since blood-*p*H data are in common logarithms, gene action may be considered additive on a logarithmic scale.

Additive gene effects are implied from the linearity of response to selection. Progress in selection is assumed to be due mostly to additive gene effects and

realized heritability was calculated to be 16.8 percent. Further information on gene action comes from analysis of variance of the T line of generation 8. Heritability based on variance due to dams (σ_D^2) was 29.0 percent compared to zero percent based on variance due to sires (σ_S^2). The difference between heritabilities based on σ_D^2 and σ_S^2 is within sampling error but gives some indication of environmental variance common to full sibs (maternal influence), allelic interactions (dominance), or nonallelic interaction (epistasis). An analysis of *p*H data from inbred PHH and PHL strains shows a within-litter variance equal to between-litter variance and indicates that maternal effects are either absent or negligible.

Response appears at first to be greater in the high line (Figure 2). However, total response is equal in TH and TL lines as judged by their divergence from contemporary control line means. Due to a source of error in classifying the phenotype of low line parents which was not encountered in classifying high line parents, possible discrepancies between selection differentials cannot be assessed.

Crosses (F_1, F_2, and backcrosses) are of limited value for the detection of directional dominance or overdominance since parental lines were not inbred. Midparents had large sampling errors, and differences would have had to have been extreme for detection. Additional crosses after the lines have become homozygous by continued inbreeding will be necessary before conclusions can be reached. At the present stage of investigation, there is no evidence for directional dominance or overdominance, nor is there any reason to believe that gene frequencies might be greater in one direction over the other in the foundation stock. Asymmetry of response is not indicated on the basis of available evidence.

Selection limits: There was no reduction in genetic variance as reflected by phenotypic variance as selection advanced. If genetic variance had become less through generation 4, response would have been nonlinear and realized heritability would decrease as selection limits were approached. No such trends are evident. By the same reasoning, if genetic variance was not reduced, then there was no increase in environmental variance since variability at the phenotypic level did not change appreciably.

Natural selection may set selection limits by causing a differential fertility of parents or a differential mortality of the young (from fertilized egg to measurement of phenotype). Loss of young or reduction of litter size should be evident as a discrepancy between expected and effective selection differentials. If natural selection were acting against extreme phenotypes, then the number of offspring contributed by the more extreme parents would be fewer than that of the less extreme parents, and differentials would be correspondingly less (see Table 2). Little, if any, difference is evident. There is furthermore no reduction in litter size, decreased viability, or increase in number of sterile matings up to the time inbreeding was initiated in generation 6.

Physiological limit may be another cause of selection limits (FALCONER and KING 1953). A skewed frequency distribution of phenotypes may indicate its existence. As seen in Figure 3, the TH and TL frequency distributions are slightly skewed but not significantly so. Another indication of physiological limit is a

reduction in genetic variance with a corresponding decline in heritability as selection limits are approached. This is contraindicated by the data. Physiological limit may be ruled out as any important cause of selection limits in this experiment.

The most probable cause of selection limits is negative correlations between different genotypes, each of which produces the desired phenotype. Blood-*p*H is a phenotype with multiple causal paths, and selection may be directed at any one or combination of causes for difference in *p*H. It is desirable to examine some of the physiological mechanisms underlying blood-*p*H as possible targets of selection for a better understanding of selection gains and associated phenomena.

Targets of selection: A possible target of selection is behavior. Mice may differ in temperament, activity (different kinds as well as total), reactions to learning situations, and in a variety of other ways. Endocrine secretion levels may account for some differences in temperament as well as differences in activity. General activity could be associated with differences in basal metabolism which in turn could be a function of thyroid activity. It has been established that inbred and F_1 hybrid mice have different levels of thyroid activity which are genetically determined and which are associated with differences in behavior (CHAI, AMIN and REINEKE 1957; CHAI 1958). Thyroid activity level in rats has been experimentally changed by selection (SUNDER 1960), and response appears to be comparable to that for blood-*p*H. Behavioral differences then could influence blood-*p*H by effecting changes in metabolic rate, or behavioral differences themselves could be an effect from another primary cause.

Gross observations in the laboratory have indicated that a behavioral difference exists between lines selected for high and low blood-*p*H. Mice (TH, TL, T, F_1, F_2, and backcrosses) were housed six to a cage and randomized as to line or cross in generations 4, 5, and 6 shortly after weaning. A frequency distribution of sampling order of mice picked at random from each cage at the time of testing for *p*H, or at the time of segregation in the case of generation 6, disclosed that mice of the TH line were predominant among the first to be sampled while mice of the TL line were more numerous among the last to be sampled. This tendency was consistent but was not statistically significant by chi-square analysis. It has been found that the last mice to be picked out of a cage, strain being constant, have a lower blood-*p*H than those first to be picked. This may be due to an accumulation of acid metabolites in the blood and subsequent lowering of *p*H. As mice become fewer in a cage and degrees of freedom for choice are reduced, the remaining mice become even more excited and difficult to capture. One would expect the effect on blood-*p*H to be nonlinear.

Ancillary evidence in favor of some sort of behavioral or metabolic difference has come from several sources. TH, TL, PHH and PHL mice were tested for a difference in general activity using exercise wheels with revolution counters (J. L. LENZ, unpublished). Activity was recorded as the number of revolutions over a given period of time. PHL mice showed greater activity than PHH mice. Differences were statistically significant. TL mice were more active than TH mice but not significantly so. Numbers were small, and the experiment is still in progress at this writing.

As a result of a recent pilot experiment, lactic acid content of the blood of the PHH and PHL strains has been found to differ significantly. Mice of the PHL strain have a higher concentration of lactic acid than PHH mice. Too few data have been collected for mice of the TH and TL lines to tell whether a similar difference exists.

High lactic acid is usually indicative of either sudden or severe exertion. It is conceivable that mice which become "excitable" or active on slight provocation would reflect a lowered *p*H of the blood as a result of accumulation of lactic acid and CO_2.

An analysis of absolute thyroid weights (left lobe plus right lobe) of TH, T and TL mice of generation 6 chosen at random showed that T ($n = 21$) > TH ($n = 18$) > TL ($n = 26$) but differences were not statistically significant.

Sex ratio: The first suspect for the association of blood-*p*H and sex ratio is genetic correlation, mediated by linkage or pleiotropy. If linkage or pleiotropy are responsible, then repeated samplings from the same foundation stock should give similar differences in sex ratio. This is subject to analysis from the data. What essentially constitutes one generation of selection from the foundation stock occurred in each generation to the next in the course of normal maintenance of the T line. Blood-*p*H determinations were made for most of the mice which were later mated. Accordingly, a correlation coefficient between midparental *p*H and sex ratio of the litter produced can be calculated. Data are available for generations 3–4; 4–5; 5–6; 6–7; and 7–8. No significant correlation was found ($r = +.08$; 22 d.f. for pooled data). A puzzling feature of the change in sex ratio is that the response appeared in generation 1 and did not increase as divergence in blood-*p*H between TH and TL lines became greater. Results were similar for PHH and PHL lines (WEIR 1953).

SUMMARY

High and low lines of mice have been established by selection for blood-*p*H. Selection was based on individual merit, and minimal inbreeding was practiced for five generations until it became evident that no further progress was possible. Brother-sister matings were then initiated, and selection was continued for two additional generations. The experiment was in part a replication of an earlier two-way selection experiment from the same heterogeneous foundation stock. The foundation stock was maintained contemporaneously to selected lines as a control. The new lines are designated TH (high) and TL (low), whereas those from the earlier experiment are known as PHH (high) and PHL (low).

Response was immediate. Maximum divergence between TH and TL lines was .069 *p*H units and was attained in the fourth generation. The rapid response and early limits to selection indicate that genetic control is by a few additive loci. Response did not diminish as the plateau was approached.

Selection differentials were greater in the TL line, but response in TH and TL lines was equal as judged by their divergence from the control line. The asymmetry is believed to be due to errors in classification of type of blood (arterial

versus venous) in choosing parents for mating in the low line and not to any real differential response.

Realized heritability was 16.8 percent for TH and TL lines combined, compared to an estimate of 14.5 percent based on intraclass correlations between full sibs of the control line. Estimates are biased in the minus direction because of some inbreeding in the foundation stock at the beginning of selection. Maternal effects on blood-*p*H appear to be absent or negligible. There is some indication of dominance and epistasis from the discrepancy between variance due to dams and variance due to sires, but differences are within sampling error.

Gene action is additive on a logarithmic scale. Blood-*p*H means from F_1, F_2, and backcrosses were equal to midparent within sampling error and there is no evidence for directional dominance or overdominance either from crosses or the nature of the selection response. Crosses of TH and TL with PHH and PHL (interselection crosses) gave blood-*p*H means midway between contemporary parental lines.

A basic difference between the two selection experiments is revealed by a comparison of arterio-venous differences. In selecting TH and TL lines, *p*H determinations were made from arterial blood compared to venous blood in the PHH and PHL strains. Selection of TH and TL lines using arterial blood had the effect of magnifying the arterio-venous difference in *p*H in the high line and reducing it in the low line. The opposite is true in PHH and PHL strains. It seems evident that different loci are involved in the two experiments.

Phenotypic variance did not decrease as selection advanced, and no reduction in genetic variance is inferred. Natural selection or a physiological limit do not appear to be limiting factors to selection. Selection limits are postulated to be due for the most part to negative correlations between multiple targets of selection. There is no clear evidence for genetic homeostasis.

A significant change in sex ratio occurred as a correlated response to selection. The TH line has a higher percentage of females; the TL line has a higher percentage of males. The change in sex ratio was opposite to that observed in PHH and PHL strains.

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THE CYTOGENETICS OF SPECIATION IN ASIATIC COTTON[1]

L. L. PHILLIPS

North Carolina State College, Raleigh, N. C.

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THE cultivated cottons are divided into two groups: the diploid Asiatic species *Gossypium arboreum* and *G. herbaceum* and the New World amphidiploids *G. hirsutum* and *G. barbadense*. The taxa within each of these groups are readily crossed, and the F_1 in each case is vigorous and fertile. F_2 and subsequent generations, however, contain either depauperate types or plants that closely resemble one of the parents.

HARLAND (1936), after considerable study on the genetics of *G. hirsutum* and *G. barbadense*, concluded that the breakdown following hybridization of these species was due to the rearrangement of their coadapted modifier systems.

SILOW (1944a) employed much the same argument to account for the specific differentiation between *G. arboreum* and *G. herbaceum*, and rejected any explanation based on chromosome structural differences between the two species.

STEPHENS, in a 1950 review citing genetic, cytological, and plant breeding evidence, indicated that multiple gene substitution alone cannot account for the differentiation in these species. He suggested that species of Gossypium differ from one another by many chromosome structural differences too small to be easily detected by cytological methods. He further set forth three cytogenetical criteria by which cotton species could be tested for this cryptic structural differentiation: (1.) Selective elimination of donor-parent genotype in interspecific backcrosses. (2.) A reduction in crossing over in interspecific crosses. (3.) Deviation from autopolyploid segregation ratios in synthetic polyploids of interspecific hybrids.

STEPHENS (1949) and LEWIS and MCFARLAND (1952) in separate studies that complemented each other demonstrated selective elimination of donor-parent genes in backcrosses of *G. hirsutum* × *barbadense* hybrids. LODEN (1950) previously had shown that recombination for the red *(R_1)*-cluster *(Cl_1)* linkage is less frequent in the *G. hirsutum* × *barbadense* hybrid than in either parent.

The present study was designed to test all three of STEPHENS' criteria in a series of crosses involving the two Asiatic diploid species *G. arboreum* and *G. herbaceum*. The genetic marker stocks used in these crosses and the alleles they carry are listed in Table 1.

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TABLE 1

Genetic constitution of marker stocks

G. arboreum				*G. herbaceum*	
SMA1		S5001		"red" *herbaceum*	
R_2^{mgs}	Red plant body	r_2	Green plant body	R_2	Red plant body
F	Tufted seed	f	Fuzzy seed	f	Fuzzy seed
*L^N	Narrow leaf	l	Broad leaf	l	Broad leaf
**cu*	Curly leaf	Cu	Non curly leaf	Cu	Non curly leaf
*Lc_1	Brown lint	lc_1	White lint	lc_1	White lint

* Linked loci.

TABLE 2

Backcross segregations of intraspecific G. arboreum *and interspecific* G. arboreum × herbaceum F_1 *hybrids*

Intraspecific SMA1 × S5001				Interspecific SMA1 × red *herb.*		
P	χ^2	Seg.	Marker*	Seg.	χ^2	P
		112	r_2 or R_2	228		
.8–.9	.039				.307	.5 –.7
		115	R_2^{mgs}	240		
		115	l	225		
.9–.95	.004				.6922	.3 –.5
		114	L^N	243		
		118	Cu	220		
.5–.7	.214				1.675	.2 –.3
		111	cu	248		
		122	lc_1	231		
.3–.5	.982				.077	.8 –.9
		107	Lc_1	237		
		124	fz	208		
.1–.2	1.942				5.778	.01–.02
		103	Fz	260		

* The *G. herbaceum* allele is listed first for each segregation.

MATERIALS AND METHODS

From the plant materials listed in Table 1 the intraspecific *G. arboreum* (SMA1 × S5001) and interspecific *G. arboreum* × *herbaceum* (SMA1 × "red" *herbaceum*) crosses were made. Upon being crossed with a suitable recessive *G. arboreum* tester stock they gave the data on gene transmission and crossing over recorded in Tables 2 and 3. Segregation frequencies from 4x intraspecific, interstrain *G. arboreum* and 4x *(G. arboreum* × *herbaceum)* are recorded in Table 4. The synthetic amphidiploids were made by using the colchicine-tragacanth method of SCHWANITZ (1949). These tetraploids are duplex for the marker genes

TABLE 3

Linkage between L^N, Cu *and* Lc_1 *in intraspecific* G. arboreum *and interspecific* G. arboreum × herbaceum F_1 *hybrids*

Intraspecific SMA1 × S5001			Interspecific SMA1 × red *herb.*	
Percent c.o.	No.	Crossover class*	No.	Percent c.o.
		Parental		
	72	*L-cu-Lc*	184	
	78	*l-Cu-lc*	172	
65.5	150		356	76.1
		Singles		
	12	*l-cu-Lc*	24	
	17	*L-Cu-lc*	19	
12.6	29		43	9.2
		Singles		
	23	*L-cu-lc*	39	
	21	*l-Cu-Lc*	28	
19.2	44		67	14.3
		Doubles		
	4	*l-cu-lc*	1	
	2	*L-Cu-Lc*	1	
2.6	6		2	0.4

Heterogeneity $\chi^2 = 26.69$, d.f. $= 3$; $P = < .01$

* Gene symbols abbreviated.

TABLE 4

Tetraploid segregation in synthetic polyploids of interspecific G. arboreum *and interspecific* G. arboreum × herbaceum F_1 *hybrids*

Combination	Anthocyanin pigmentation			Leaf-shape				
	R_2R_2	R_2r_2	r_2r_2	L^NL^N	L^Nl	ll	L^LL^L	L^Ll	ll
Intraspecific									
4x(SMA1-S5001) × Upland	33	156	42	145*		30	..	..	..
4x(S5133-S5001) × Upland	..	..	..	..	..	..	52	201	46
χ^2 (1:4:1 or 5:1)	1.129			.026			.399		
P	(2) .5–.7			(1) .8–.9			(2) .8–.9		
Interspecific									
4x(SMA1-red *herb.*) × Upland	76	308	67	406*		46	..	..	..
4x(S5133-red *herb.*) × Upland	..	..	..	..	..	..	47	418	56
χ^2 (1:4:1 or 5:1)	1.282			13.678			43.525		
P	(2) .5–.7			(1) < .01			(2) < .01		

* The Narrow (L^NL^N) and intermediate (L^Nl) leaf-shape classes were indistinguishable; hence leaf-shape was scored as a two-class segregation.

listed in Table 1 and are therefore expected to segregate 5:1 or 1:4:1 for the markers if random chromosome pairing and disjunction are assumed. Preferential pairing will decrease segregation (widen ratio); chromatid segregation will tend to increase it.

Ideally the intraspecific and interspecific hybrids should have been tested with both *G. arboreum* and *G. herbaceum* stocks, but the suitable recessive alleles were available only in *G. arboreum;* hence the backcross was made in only one direction.

RESULTS

Gene transmission in diploid F_1 × G. arboreum tester: As can be seen in Table 2 only one of the segregations studied deviated significantly from the expected 1:1 backcross ratio. In this, the segregation for amount and distribution of seed fuzz, there was considerable elimination of the *G. herbaceum* factor *(f)*. In the remaining four segregating families there was a nonsignificant discrimination against gametic transmission and/or zygotic survival of *G. herbaceum* genes.

Genetic recombination in intra- and interspecific F_1 hybrids: Genetic recombination within the three point linkage group delimited by the leaf shape and lint color loci and including the curly locus was significantly reduced in the interspecific hybrid as compared with the intraspecific F_1. Thus crossing over between L^N and Lc_1 was 37.0 percent in the intra-*arboreum* cross and reduced to 24.3 percent in the interspecific hybrid. Silow (1944b) found a reduction in crossing over between leaf shape and lint color of a similar magnitude in interspecific hybrids of *G. arboreum* and *G. herbaceum* as compared to their parents.

Segregation in tetraploid G. arboreum × herbaceum: Segregating progenies from the cross 4x *(G. arboreum* SMA1 × "red" *herbaceum)* × commercial Upland were scored for leaf shape and anthocyanin pigmentation (Table 4). The remaining markers could not be scored because of lack of a suitable tetraploid tester. Segregation for R_2 was 384 red:67 green (5.31:1), very close to the autotetraploid 5:1 ratio. The segregation for leaf shape was significantly different from that for anthocyanin coloration, being 406 narrow:46 broad or 8.83:1. The segregations for these two markers from *G. arboreum* × *herbaceum* amphidiploids, along with ratios for three other markers, have been reported previously by Gerstel and Phillips (1957). Four of the five segregations recorded in that report were near the autotetraploid ratio (5:1 or 1:4:1) while the fifth, that for leaf shape, was 9.8:1 (49 narrow:5 broad). Because of the small population scored for leaf shape this deviation from a 5:1 ratio was not significant. The present segregation of 8.83:1 based on a population of 452 plants is different and, as will be brought out below, indicative of chromosome structural divergence of *G. arboreum* and *G. herbaceum.*

DISCUSSION

Divergence between *G. arboreum* and *G. herbaceum* for the chromosome segment containing the leaf-shape, curly and lint-color loci is indicated by all three

of the criteria applied. The test for gene transmission is less decisive than the remaining two since the differences here, though indicating a trend toward donor elimination for all three loci, were nonsignificant. The deviations for genetic recombination and tetraploid segregation were significant.

A decrease in crossing over in an interspecific hybrid can be explained on a structural basis, i.e., less chromosome homology will result in reduction of chiasmata, or on the assumption that gametic or zygotic selection eliminates many of the non-coadaptive gene arrangements effected by crossing over. But if this latter explanation was applicable, one would expect that gametic elimination would be proportional to the total chromosome length (and therefore number of genes) involved in recombination (i.e., a product of meiosis in which 25 percent of the total chromosome length was involved in recombination events would be more subject to elimination than a meiotic product in which only 5 percent of the total chromosome length was affected by crossing over). If depression in over-all recombination percentage is a result of gametic selection and the foregoing assumptions are valid one would expect that the percentage reduction in recombination from control to the interspecific hybrid would be greater between the proximal locus (gene order, relative to centromere, is not known) of the linkage group and *Cu* than between the distal locus and *Cu*; this being due to greater survival value of gametes with a lesser amount of recombined chromatin. That this is not the case is indicated by the fact that in the interspecific hybrid the reduction in crossing over between *L* and *Cu* (27%) was very similar to the reduction between *Cu* and *Lc* (26%). Further, interference is negligible in the intraspecific cross (coinc. = 1.1) and significant in the interspecific cross (coinc. = .33). Since it is unlikely that selective elimination would influence interference (double crossovers would involve no more, and probably less, chromatin than singles) this is additional evidence that a reduction in chiasmata frequency is responsible for the observed decrease in recombination.

Since this test of the relative importance of gametic elimination in accounting for observed gene frequencies in interspecific backcrosses and F_2's involves something less than five percent of the total chromosome length, it cannot be considered a particularly critical method of evaluation. The data (especially those for double crossovers) do indicate, however, that selective gametic elimination is not a major factor in the reduction of genetic recombination in the interspecific hybrid.

An explanation attributing reduction in crossing over in the interspecific hybrid to reduction in chiasmata frequency between incompletely homologous chromosomes seems to fit the data better than does any other explanation. The significant decrease in single and double crossovers would be expected and predictable on this basis.

The tetraploid segregation for L^v was scored in 1958. Since the 8.83:1 ratio for this allele was quite different from the segregations scored for other loci in synthetic amphidiploids of the Asiatic species (which clustered around 5:1) it seemed desirable to verify this value by studying the tetraploid segregation of another leaf-shape allele (L^L) of the Asiatic leaf-shape series. It was also deemed desirable to determine whether the leaf-shape locus was on one of the chromosomes for

which *G. arboreum* and *G. herbaceum* differ in end arrangement (GERSTEL 1953). The segregation of L^L (9.12:1, Table 4) proved to be very similar to that for L^N. There was no correlation between the leaf-shape locus and the semisterility or the cytologically detectable quadrivalent associated with the *G. arboreum-herbaceum* translocation.

The 8.98:1 segregation for leaf-shape (L^N and L^L segregations combined) must be the result of preferential pairing brought about by chromosome structural differences. It is improbable that genic differences could result in such a significant deviation from the autotetraploid 5:1 ratio.

In recent years several investigators (MICHIE 1953; WALLACE 1953 and 1958) have evolved the "affinity" concept to explain the apparent linkage of genes known to be independent. Thus loci can exhibit "quasi-linkage" due to differential affinity of centromeres resulting in their non random assortment to the poles at metaphase I. Such a mechanism could account, at least in part, for the preponderance of parental type recovered in backcrosses, F_2 and F_3 progenies of *G. arboreum* × *herbaceum* hybrids. There is no way of determining from the present data the importance of affinity relative to the elimination of gametes and zygotes imbalanced by non-coadaptive chromosomal arrangements, though the presence of depauperate types in early generation *G. arboreum* × *herbaceum* progenies would seem to indicate that the later phenomenon is of some importance: the possible role of affinity remains a moot question.

An interesting parallel to the situation described above for *G. arboreum* × *herbaceum* hybrids is found in early-generation progenies of *G. arboreum* or *G. herbaceum* × *anomalum*. Here sterility is greater and F_2 breakdown more drastic. The cytological differences in either of these crosses are quite obvious rather than "cryptic" as in the *G. arboreum* × *herbaceum* hybrid. An average of nine bivalents are seen in F_1 *G. arboreum* × *anomalum* or *G. herbaceum* × *anomalum* (SKOVSTED 1937), and tetraploid segregation for several markers average 50:1 (GERSTEL and PHILLIPS 1957). As the data presented in this paper indicate, and as STEPHENS (1950) has pointed out, there is every reason to assume that the differences between *G. arboreum* and *G. herbaceum* on the one hand and between the Asiatics and *G. anomalum* on the other differ in degree rather than kind.

SUMMARY

The degree and nature of speciation in the Asiatic cottons, *G. arboreum* and *G. herbaceum* have been determined by assessing the genetic behavior of the linked loci *L*, *Cu*, and *Lc*, and two other loci in the light of gene transmission, genetic recombination, and tetraploid segregation in their interspecific hybrids.

A nonsignificant degree of selective elimination of donor parent genotype in backcross progenies was noted for each of the linked loci. There was a significant amount of elimination found for one of the two other loci.

Crossing over within the *L-Cu-Lc*, linkage and segregation for leaf shape were both significantly altered from expected in the interspecific hybrid.

The results are explained on the basis of cryptic structural differentiation of the chromosomes of the two species.

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EXPERIMENTS ON HYBRID SUPERIORITY IN DROSOPHILA MELANOGASTER. II. RATE OF DEVELOPMENT FROM EGG HATCHING TO ECLOSION

GERT BONNIER

Institute of Genetics, University of Stockholm, Stockholm, Sweden

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HYBRID superiority with regard to egg laying capacity and larval survival in *Drosophila melanogaster* was discussed in a previous paper (BONNIER 1961) where also a general discussion was given. That investigation is, in the present paper, supplemented by a comparison of the rate of development of hybrids and nonhybrids. The material for the present study is the same as that used before: for all details, reference is therefore made to the previous paper. Here it may suffice to remember that the source is three unrelated wild type stocks, which were named E, K, and T. Three populations e, k, t, were synthesized in such a way that they, on the one hand, were homozygous for the three major chromosomes, and that, on the other hand, these chromosomes as well as the Y chromosome in e were derived from E, in k were derived from K, and in t were derived from T.

To produce the homozygous populations e, k, t, used in the present investigation flies from the last generation described in the previous study were mass propagated for two more generations; the resulting flies were made parents of the larvae, the development of which was to be studied. Among the stock flies the investigation makes use of pures (i.e. larvae from E, K, T) and of hybrids (i.e. F_1 larvae from all six types of crosses between E, K, T including reciprocals). Likewise, among homozygotes the investigation makes use of pures (i.e. larvae from e, k, t) and of hybrids (i.e. F_1 larvae from all six types of crosses between e, k, and t). There were, hence, three pure and six hybrid genotypes, or in all nine genotypes, from stock flies and an equal number of genotypes from homozygous flies.

Petri dishes with black food were placed in cages with flies and kept in a laboratory room which was especially arranged as an incubator room at 25°C. After 16 hours of oviposition and 28 more hours of incubation, freshly hatched larvae were collected and transferred to vials with ordinary food, about 12 ml per vial. Three different experiments were performed in which all 18 genotypes were used:

Experiment 1: 200 larvae of each genotype were put into each of four vials which were incubated at 25°C.

Experiment 2: 100 larvae of each genotype were put into each of four vials and to each of these vials were added 100 larvae from flies of the white *(w)* stock; incubation at 25°C.

Experiment 3: 200 larvae of each genotype were put into each of four vials which were incubated at 30°C.

With the aid of a table of random numbers all vials were randomly arranged. The vials belonging to experiment 1 and 2 (incubated at 25°C) were kept in this order in trays in the same laboratory room as that which was used as an incubator room for the cages. The vials belonging to experiment 3 were kept in their random order in trays put into special incubator at 30°C. As may be found, the total number of vials for each of the three experiments was 36 for larvae from stock flies and 36 for larvae from homozygous flies; the grand total was, thus, 216 vials each with 200 larvae, or a grand total of 43,200 larvae.

The trays with the vials were left undisturbed until eclosion began. Thereafter the vials were emptied every fourth hour and the adults were counted for each vial separately. The two sexes were not counted separately, but, in experiment 2 wild type flies and white eyed flies were counted separately. The white flies are, however, not included in the figures and the analyses below.

The time which 50 percent of the larvae needed to reach eclosion is used as an appropriate measurement of the rate of development. The four-hour period is used as the unit of time with the zero point at 7 days 14 hours after the beginning of the oviposition of the females which produced the larvae. The number of four-hour periods for the 50 percent eclosion was determined for each vial by means of a linear interpolation between the last four-hour period, when less than 50 percent of the larvae were recovered as adults, and the first, when more than 50 percent were recovered as adults. The number of four-hour periods thus found is used as the rate of development in that vial. The average from the four vials of each genotype is then used as the measurement for this genotype.

RESULTS

Table 1 shows the numbers of larvae which were recovered as adult flies. These numbers are expressed as percentages of the numbers of larvae collected. The

TABLE 1

Percentages of larvae recovered as adults

	Larvae from stock flies					Larvae from homozygous flies				
	Genotype of		Experiment			Genotype of		Experiment		
	mother	father	1	2	3	mother	father	1	2	3
Pures	E	E	92.9	88.0	76.5	e	e	94.3	90.2	84.9
	K	K	96.4	92.8	86.8	k	k	92.8	86.3	79.8
	T	T	96.9	94.3	90.0	t	t	90.4	93.0	81.0
Hybrids	E	K	94.3	97.3	94.6	e	k	90.6	91.8	88.1
	K	E	94.0	100.0	87.1	k	e	91.1	92.5	85.6
	E	T	93.9	91.8	84.5	e	t	93.8	95.0	83.9
	T	E	92.9	94.5	85.6	t	e	94.3	94.3	85.1
	K	T	95.3	96.8	91.3	k	t	95.9	100.0	82.4
	T	K	93.9	96.5	91.4	t	k	86.9	87.8	89.8

The figures belonging to the four vials of each separate genotype are pooled. For each separate genotype, therefore, the percentages shown are based on 800 larvae in experiments 1 and 3, and on 400 larvae in experiment 2.

table does not show the percentage for each separate vial, but percentages pooled for each separate genotype.

The time needed for 50 percent of the larvae to reach the adult stage may be computed in two different ways, and, as there is no *a priori* reason for saying that the one gives a better picture of the true situation than the other, both ways are applied in Table 2. These are:

a. The 50 percent is based on the total number of larvae collected. As this number is 200 per vial in experiments 1 and 3, 50 percent means that 100 adult flies should have emerged. In experiment 2 the number of larvae collected per vial is 100, and 50 percent means that 50 adults should have emerged.

b. The 50 percent is based on the total number of larvae which actually emerged as adults in the different vials (see Table 1).

The results are shown in Table 2. But as the only comparisons of major interest are those between pures and hybrids, the results in Table 3 are pooled for all pures from stock flies (including three genotypes, i.e. 12 vials), and the same is done with all pures from homozygous flies. Likewise, the results of all hybrids from stock flies and homozygous flies respectively (including each six genotypes, i.e. 24 vials) are pooled. The estimate of the significance level, P, is thus based on one degree of freedom for pures *versus* hybrids and seven degrees of freedom for within (two within pures and five within hybrids).

TABLE 2

Time needed for 50 percent of pure and hybrid larvae to recover as adults

	Larvae from stock flies					Larvae from homozygous flies				
	Genotype of		Experiment			Genotype of		Experiment		
	mother	father	1	2	3	mother	father	1	2	3
a. Pures	E	E	23.6	25.4	19.3	e	e	25.6	22.3	17.2
	K	K	25.1	21.5	18.1	k	k	27.0	24.9	21.9
	T	T	23.3	22.6	16.1	t	t	26.1	23.2	17.2
Hybrids	E	K	23.3	19.6	13.7	e	k	23.1	20.5	15.8
	K	E	21.1	19.8	15.6	k	e	26.0	21.9	17.6
	E	T	25.3	19.6	14.4	e	t	27.6	21.2	18.3
	T	E	24.0	20.2	16.3	t	e	25.5	22.8	17.1
	K	T	24.8	21.3	16.1	k	t	24.2	23.0	18.3
	T	K	25.4	19.0	15.1	t	k	23.4	23.3	16.2
b. Pures	E	E	23.4	24.9	18.2	e	e	25.2	21.6	16.1
	K	K	24.9	21.3	17.5	k	k	26.5	24.2	20.5
	T	T	23.1	22.2	15.5	t	t	25.4	22.7	16.1
Hybrids	E	K	22.9	19.5	13.5	e	k	22.5	20.2	15.3
	K	E	20.9	19.8	15.0	k	e	25.5	21.5	16.7
	E	T	25.1	19.4	14.1	e	t	27.0	21.0	17.0
	T	E	23.6	19.9	15.4	t	e	25.2	22.6	16.3
	K	T	24.5	21.3	15.6	k	t	23.8	22.9	17.2
	T	K	25.0	19.0	14.7	t	k	22.5	22.1	15.9

Figures show averages for the four vials of each genotype. Time measured in periods of four hours with zero point at 7 days, 14 hours after beginning of the 16 hours of oviposition of the females which produced the larvae. In a, the 50 percent values are based on the total number of larvae collected; in b, the 50 percent values are based on the total number of larvae actually recovered as adults.

TABLE 3

Average time needed for 50 percent of pure and hybrid larvae to recover as adults

	Larvae from stock flies Experiment					Larvae from homozygous flies Experiment		
	1	2	P	3	P	1	2	3
a. Pures	23.99	23.17		17.83		26.20	23.43	18.76
			0.01–0.005		0.025–0.01			
Hybrids	23.98	19.91		15.18		24.96	22.10	17.21
b. Pures	23.78	22.78		17.05		25.66	22.83	17.53
			0.01–0.005		0.025–0.01			
Hybrids	23.65	19.79		14.71		24.41	21.71	16.39

The averages are computed from the totals and not as averages of the averages given in Table 2; the small discrepancies are due to this cause. The significance probability, P, of the difference between pures and hybrids is less than 0.05 only for larvae from stock flies, and, among them, only in experiments 2 and 3. P is, therefore, only shown for these two cases. See Table 2 for meaning of a and b.

As may be seen from Table 3 the hybrids in all cases develop faster than the pure ones. The differences are, however, clearly significant only in the case of larvae from stock flies, and for them only for experiments 2 and 3.

DISCUSSION

It is an often made observation that if one lets different numbers of larvae of *Drosophila melanogaster* develop within vials with equal amounts of food, development will be slower the more larvae there are (at least when the number of larvae surpasses a certain minimum value). This difference in rate of development is certainly a composite effect of several causes. Crowding may in itself retard development and so may other kinds of environmental influences. But it is obvious that also the competing ability of the individual larva may enhance or decrease the rate of development of this particular larva. If one, now, has a series of vials, all with equal numbers of larvae and all with equal amounts of food and if all larvae within each separate vial possess equal competing abilities, then comparisons between the rate of development of larvae occupying different vials cannot justify any conclusive statements concerning their relative competing abilities. In a vial with larvae of a high competing ability each separate larva, though itself strong as a competitor, has to withstand a strong competition pressure from the rest of the larvae; and in a vial with larvae of a low competing ability each larvae, though itself weak as a competitor, has only a weak competition pressure to withstand from the rest of the larvae. The relative magnitude of the competition pressure which the individual larvae has to withstand may thus be equal, irrespective of the absolute magnitude of this pressure.

A simple mathematical model may be made. Let the number of larvae in the vials be n and the competing ability of the larvae within a certain vial be x. The relative pressure which the separate larvae have to withstand is then about $nx/x = n$, i.e. a value which is independent of the value of x. The fact that in experiment 1 (Table 3) no difference was found between pures and hybrids among larvae from stock flies, hence, does not permit any definite conclusions to be

drawn concerning hybrid superiority or nonsuperiority. Similarly, the greater (though statistically nonsignificant) speed of development of hybrid than of pure larvae from homozygous flies, does not permit any conclusions of that kind. The situation is, however, different in experiment 2. Here one half of the larvae were taken from one kind of the wild type flies studied and the other half were taken from the white stock. If the competing ability of each separate white larva is z, it is seen that the relative pressure which the separate wild type larvae have to withstand is about $n/2(x+z)/x = n/2(1+z/x)$, and if $z \neq x$, this value is neither independent of x nor of z. The net gain which one will get when comparing experiment 1 and experiment 2, i.e. the difference between the results from these two types of experiments, is then $n - n/2(1+z/x) = n/2(1-z/x)$. If the competing ability of the wild type larvae in some other vial is y instead of x, the net gain will similarly be $n/2(1-z/y)$. If the former gain is larger than the latter, i.e. if $n/2(1-z/x) > n/2(1-z/y)$, it follows that $x > y$.

The differences between the observed times in experiments 1 and 2 in Table 3 may be taken as corresponding to the mathematical gains just derived. They are (when using only method a of computation, as methods a and b give very similar results):

	larvae from stock flies	larvae from homozygous flies
pure larvae	$23.99 - 23.17 = 0.82$	$26.20 - 23.43 = 2.77$
hybrid larvae	$23.98 - 19.91 = 4.07$	$24.96 - 22.10 = 2.86$

In the case of larvae from stock flies the gain is several times larger for hybrids than for pures. It was stressed above that the rate of development may be influenced by many different causes. But the largeness of the difference between hybrids and pures makes it, in spite of this, rather safe to conclude that the hybrids are superior to the pures with regard to competing ability. In the case of larvae from homozygous flies no such superiority is observed. But with the fact in mind that, according to Table 3, the difference between hybrids and pures in experiment 2 was without statistical significance, no hybrid superiority was to be expected among larvae from homozygous flies.

From these results one would also expect to find that, in the case of larvae from stock flies, the white larvae would develop relatively slower in competition with wild type hybrids than in competition with wild type pures but that probably no significant differences would be observed in the case of larvae from homozygous flies. As Table 4 shows, there is a very good agreement with these expectations.

With regard to larvae from stock flies, Table 2 shows also (experiment 3) that hybrids resist 30°C better than do pure ones.

In the previous paper (BONNIER 1961), the chief interest was focused on the stock flies. It was argued that, as the stocks used were unrelated, selection could hardly have had any influence on the occurrence of hybrid superiority. It seemed, therefore, simplest to assume that overdominance was responsible for this superiority, which was observed for egg laying capacity and for larval survival. In the

TABLE 4

Competition between white and wild type larvae

	Larvae from stock flies			Larvae from homozygous flies		
	Wild type larvae competing with the white larvae			Wild type larvae competing with the white larvae		
	Genotype of mother	father	Total number of white flies	Genotype of mother	father	Total number of white flies
Pures	E	E	143	e	e	137
	K	K	196	k	k	226
	T	T	213	t	t	97
Average			184			153
Hybrids	E	K	8	e	k	42
	K	E	28	k	e	89
	E	T	98	e	t	107
	T	E	88	t	e	162
	K	T	76	k	t	170
	T	K	28	t	k	107
Average			54			113
Difference between the two averages			130			40
P for difference			0.005–0.001			> 0.2

The figures show the number of white flies (totals from four vials, each with 100 white larvae) which had emerged at the time when 50 percent of the 400 wild type larvae (i.e., 200 wild type larvae) competing with the white ones, also had emerged as adults.

present study hybrid superiority is observed for competing ability of the larvae. The new results thus agree, with the previous ones and support, consequently, also the conclusions drawn concerning the overdominance hypothesis.

In the previous paper it was also found that the difference between hybrids and pures was more pronounced for stock flies than for homozygous flies. The reason this difference, in the present study, is so much less pronounced for larvae from homozygous flies is not known.

For a more general discussion of the problem of hybrid superiority reference is made to the previous paper.

SUMMARY

1. In a previous paper (under common head title with the present one) a study was made on hybrid superiority with regard to egg laying capacity and to larval survival. The material consisted of three unrelated wild type stocks and of three homozygous populations derived from each one of the wild type stocks. The experiments were performed with pure individuals (i.e. with individuals taken directly from the stocks and from the homozygous populations respectively) and with *hybrid* individuals (i.e. F_1 individuals after the six possible crosses between pure stock flies and between pure homozygous flies respectively).

2. The present paper describes experiments on the rate of development of larvae produced by flies used in the previous experiments. With regard to larvae from stock flies, the results indicate that the hybrids are superior to pure ones: the hybrids have a better competing ability and they resist the stress of 30°C better than the pure ones. Because of this agreement with the earlier results it is

concluded that the new results, as far as they go, strengthen the conclusions drawn earlier; namely, that they support the overdominance hypothesis.

3. The hybrid superiority was, in the previous paper, found to be more pronounced for stock flies than for flies from homozygous populations. The superiority of hybrid larvae from homozygous flies is, in the present paper, very faintly expressed. The reason for this is not known.

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LITERATURE CITED

BONNIER, G., 1961 Experiments on hybrid superiority in *Drosophila melanogaster*. I. Egg laying capacity and larval survival. Genetics **46**: 9–24.

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